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# Ecological Segregation Between Longnose and Blacknose Dace (Genus *Rhinichthys*) in the Mink River, Manitoba

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GIBBONS, J. R. H., AND J. H. GEE. Ecological segregation between longnose and blacknose dace (genus *Rhinichthys*) in the Mink River, Manitoba. J. Fish. Res. Bd. Canada 29: 1245–1252.

Longnose and blacknose dace occur in greatest abundance in the middle zone of the Mink River, and segregation in environment occupied is marked. Newly emerged longnose fry were found in stream margins, but by the middle of their first summer had moved into fast riffles (>45 cm/sec) where they completed their life cycle. Blacknose fry also occupied stream margins, remaining there for up to one year. Thereafter they were found mostly in channels of moderate current. Coexistence of fry of the two species in stream margins is minimized by earlier hatching of longnose dace. Diet of longnose and blacknose dace, consisting of aquatic insect larvae and nymphs, was strikingly similar.

GIBBONS, J. R. H., AND J. H. GEE. Ecological segregation between longnose and blacknose dace (genus *Rhinichthys*) in the Mink River, Manitoba. J. Fish. Res. Bd. Canada 29: 1245–1252.

Le goujon à long nez et le goujon à nez noir sont le plus abondants dans la zone centrale de la rivière Mink, et la ségrégation de l'habitat occupé est très marquée. Les alevins de goujon à long nez, peu après leur émergement, se tiennent près des rives, mais, vers le milieu de leur premier été, se déplacent vers les radiers à courant rapide (>45 cm sec), où ils complètent leur cycle vital. Les alevins de goujon à nez noir occupent également les rives du cours d'eau, y demeurant jusqu'à une année. Par la suite, on les trouve surtout dans les chenaux à courant modéré. L'éclosion hâtive du goujon à long nez réduit la période de coexistence des deux espèces près des rives. Le régime alimentaire, constitué surtout de larves et de nymphes d'insectes aquatiques, est remarquablement semblable chez les deux espèces.

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THE longnose dace, *Rhinichthys cataractae*, and the western blacknose dace, *R. atratulus meleagris*, are cyprinid fishes that are sympatric in many water systems (Hubbs and Lagler 1964). This study investigates sympatric populations of dace in the Mink River, Man., with respect to the principle of Gause (1934), which states that no two species can coexist if they make similar ecological demands on limited resources of their environment.

Slight ecological differences between closely related species are often magnified in sympatry due to competitive interaction. Termed interactive segregation by Brian (1956), the subject has been reviewed by Miller (1967) and specifically reviewed for fishes by Nilsson (1967). Such segregation can occur with respect to food, space, and spawning sites. Requirements for spawning of both species are different (Bartnik 1970). This study provides information on distribution of both species along the length of the Mink River, their abundance in particular environments, and on food consumed to determine the degree of ecological segregation.

## Materials and Methods

The Mink River originates in the Duck Mountains, southwestern Manitoba, and flows eastwards to Dauphin Lake. Volume of flow varied in 1970 from 4.120 $m^3$/sec in May to 0.015 $m^3$/sec in August. It was divided in this study into upper, middle, and lower zones (7, 15, and 23 km in length; 4, 7, and 3 m/km mean gradient). The upper zone contained beaver dams, had mostly a silt-sand bottom, and dense overhanging vegetation (tall grasses, willow, and poplar) occurred along its length. The middle zone had similar cover and consisted of pools, channels, and riffles, with a substrate varying from sand to gravel and rocks. The lower zone was exposed with little cover as this section had been straightened and diked to prevent flooding. This zone is relatively unproductive in terms of both invertebrates and fishes. For further details, see Gibbons (MS 1971).

Dace were collected monthly from May to October, 1970, from all possible places in the river to determine their distribution along its length. Seven environments were arbitrarily defined (Table 1) and collections of dace were made in each to estimate densities of all age groups within parts of the stream occupied.

Prior to making a collection in any environment, except a riffle, the area to be sampled (5–25 $m^2$) was enclosed using barrier nets (3.5-mm diameter mesh) and fishes were captured with repeated seine hauls. In riffles, fishes were taken by kicking the substrate vigorously and driving fishes into a seine net held downstream. The seine (3.5-mm diameter mesh) had a bag 1.5 m deep and was 3 m wide. Using the above methods, seining was repeated in an area until no fishes were captured on three successive attempts. After being anesthetised in M.S. 222, dace were measured to the nearest millimeter fork length (for aging by length frequency), sexed (adults only), and then released.

Dace for digestive tract analysis were collected monthly from May to October. Each species was divided into two groups; those in their first year and those older. At least 15 fish were examined for each group. They were preserved in 10% formalin within 10 min of capture, and later transferred to 40% isopropyl alcohol. Prior to dissection fish were weighed (± 0.025 g) and measured to the nearest millimeter fork length. Stomach contents were identified to family, counted, and the total weight of each family for the group was measured (± 0.001 g).

Surber samples from 0.93 $m^2$ of substrate were taken monthly from June to September to obtain the frequency of food items in a small rock riffle and a medium channel. Organisms so collected were preserved in 40% isopropyl alcohol, identified to family, counted, and the total weight of each measured (± 0.001 g). Forage ratios (Usinger 1956) were calculated for two age-groups (0 and $1^+$ years) of each species using the numerical percentage of food items present in benthic samples and in digestive tracts. Benthic samples from a medium channel were compared to stomach contents of blacknose dace collected from such an environment while samples from a small rock riffle were used for longnose dace.

TABLE 1. Description of environments in the Mink River.

| | Environment | | | | | | |
|---|---|---|---|---|---|---|---|
| | Stream margin | Pool slow channel | Medium channel | Shallow fast channel | Gravel riffle | Small rock riffle | Large rock riffle |
| Surface velocity (*cm/sec*) | 0–15 | 0–15 | 15–30 | 25–45 | 45–100 | 45–100 | 45–120 |
| Substrate composition | | | | | | | |
| Silt-sand, <0.2 cm diam | 90%+ | 30%+ | 10%+ | 5%+ | | | |
| Gravel, 0.2–5 | | 10%+ | 40%+ | 30%+ | 75%+ | | |
| Small rocks, 5–15 | | 20%+ | 10%+ | 20%+ | | 75%+ | |
| Large rocks, >15 | | | | | | | 75%+ |
| Depth range (*cm*) | 7–25 | 35–130 | 30–50 | 10–35 | 7–25 | 7–35 | 10–35 |

Coefficients of similarity of food (Whittaker and Fairbanks 1958) were calculated to compare the diet of different size groups within or between species, using the percentage weight contribution of each family, and applied to the diet of: (a) fish of the two species age 0 years, (b) fish of the two species age $1^+$ years, (c) fish of the same species from the two above age-groups.

## Results

### Spatial Distribution

Longnose and blacknose dace were abundant in the middle zone and rare in the upper and lower zones as follows:

| | Zone | | |
|---|---|---|---|
| | Upper | Middle | Lower |
| Area sampled ($m^2$) | 164 | 2,022 | 794 |
| | *Density (no./10 $m^2$)* | | |
| Longnose dace | 0.4 | 8.8 | 1.3 |
| Blacknose dace | 0.2 | 11.9 | 0.3 |

Within the middle zone the two species generally occupied different environments.

Fry of both species were abundant in stream margins in July (Fig. 1A). Here, longnose were larger than blacknose fry (17–28 mm and 11–20 mm, respectively). Lower densities of larger longnose fry (25–39 mm) were encountered in gravel and small and large rock riffles.

In August and September, blacknose fry remained abundant in stream margins, whereas nearly all longnose fry had moved mainly into gravel and small rock riffles. This transition took place at lengths between 25 and 30 mm. Sampling in October revealed that longnose fry were mainly in riffles while blacknose were in stream margins and slow channels.

At the end of their first year, in May and June, dace had a distribution similar to that of fry in August and September (Fig. 1B), but during July and August longnose juveniles, as they increased in size, occupied mainly small and large rock riffles, respectively. At this time blacknose juveniles showed a shift to medium and fast channels, which occurred at a fork length of about 45 mm. In October, blacknose juveniles were found only in pools and slow channels (including beaver ponds) while longnose were collected from under flat stones in large rock riffles.

From length frequency, longnose are mature at age 2, but the majority of blacknose do not spawn until age 3 (Gibbons MS 1971). In their second year, juvenile blacknose were found mainly in medium and slow channels in May and June, while from July to September they were most abundant in medium and fast channels, a situation similar to that of first year juveniles that have left stream margins.

Adult longnose males, in May and June, were most abundant in small rock riffles (Fig. 2A). Females were taken mainly in large rock riffles while lower densities of females, most of which were ripe, were taken in small rock riffles. In late June these differences in distribution of the sexes were reduced, and from July to September both sexes were abundant in large rock riffles. In October, adult longnose were taken from crevices between stones in large rock riffles.

In May and early June, blacknose males were most abundant in medium and fast channels (Fig. 2B). Females were found mainly in slow channels. Many of those taken in medium and fast channels were gravid. By late June (Fig. 2B), differences between males and females in environment occupied were greatly reduced.

In late June and early July, a large proportion of the adult blacknose taken were extremely thin and emaciated in appearance. Furthermore, their numbers were greatly reduced in the latter month. It was therefore concluded that the majority had died after spawning.

### Diet

Species consumed by longnose and blacknose dace in their first year were similar in quality, with Tendipedidae, Hydropsychidae, and Baetidae making the greatest contribution of any family by weight (Fig. 3). The quantity of each item consumed varied throughout the year and differed somewhat between species.

Older longnose and blacknose dace also consumed similar items of food, consisting of Tendipedidae, Hydropsychidae, Baetidae, Limnephilidae, and Tipulidae. Ephemeridae, eaten by blacknose dace, were the only exception (Fig. 3). In both species, Hydropsychidae accounted for over 50% of the weight of the diet from early June to September, with Baetidae of secondary importance in late July and early August. But in May and October longnose dace continued to eat Hydropsychidae as the major item, while blacknose dace consumed mainly Ephemeridae and Tipulidae.

Baetidae were highly foraged by each age-class in both species, as were Tendipedidae with the exception of older blacknose (Fig. 4). Ephemeridae had high values for blacknose only as this family was not encountered in riffles. High forage ratios indicate that these families were either most preferable or most available. The relatively high occurrence of

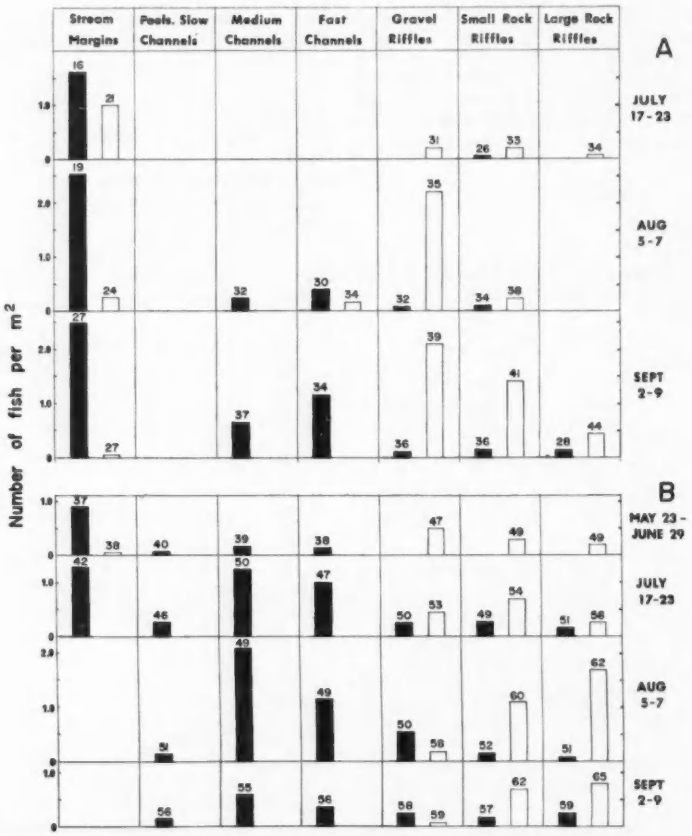


FIG. 1. Densities of longnose (open) and blacknose (solid) fry (A) and juveniles (B) in seven environments of the Mink River. Figures above bars represent mean fork length of fish.

Baetidae in drift sampling (Gibbons MS 1971) indicates the latter for this family.

Coefficients of similarity of food revealed that the diet of longnose and blacknose dace age 0 years was similar, as was that of age 1+ dace, especially in June, July, and August (Table 2). Diet of each age-group within both species was also similar except for blacknose dace in October and May.

## Discussion

Longnose and blacknose dace are most abundant in the middle zone of the Mink River. Ecological segregation results from temporal isolation of fry, which initially occupy a similar environment, and by spatial isolation in which older longnose occupy faster riffle environments and blacknose occupy channels of moderate water velocity. However, diet of the two species is similar during most of the growing season.

Bartnik (1970) found that longnose dace started spawning some 2 weeks before blacknose dace, although there was some overlap in spawning time. With a similar rate of egg development in both species (Gee unpublished data), most longnose, therefore, emerge from the substrate earlier and are present in stream margins before blacknose fry, reducing the time of coexistence in this environment. Longnose were between 25 and 30 mm when they left the margins for fast water (>45 cm/sec). Gee (1972) has found that at lengths between 18 and 27 mm this species can make its greatest adjustment of buoyancy, from near neutral to highly negative. Jones (1957), Nelson (1961), and Gee (1968) have

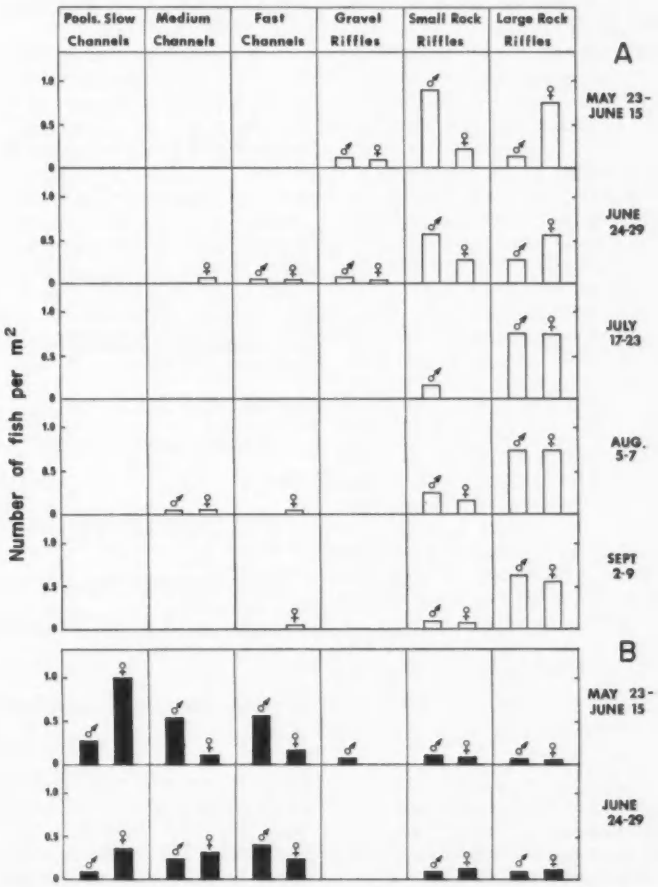


FIG. 2. Densities of adult male and female longnose (A) and blacknose (B) dace in seven environments of the Mink River. No adult dace were captured in the stream margins environment.

all noted the advantages of negative buoyancy in fast water, which is attained by reducing swimbladder volume. Blacknose dace at a similar size are incapable of making a significant adjustment of swimbladder volume and remain at near neutral buoyancy (Gee 1972). The majority remained in stream margins for about a year until they reached about 45 mm fork length. A smaller proportion were found in moderate current (20–45 cm/sec) at lengths of 25–45 mm. Gee (1972) has found that at 27–40 mm, blacknose dace become capable of adjusting buoyancy from neutral to negative by decreasing swimbladder volume. The extent of adjustment increases with size in this species.

Segregation of longnose and blacknose dace extends to spawning sites. Bartnik (1970) found redds of longnose dace in small rock riffles, while those of blacknose were in medium and fast channels. We found that within each species the two sexes generally occupied different environments in May and early June, except that ripe females were found with males. Males of both species are territorial, each defending an area with a potential nesting site prior to spawning and with eggs after spawning. Females are nonaggressive. Prior to spawning, males of both species show aggression to their respective males and females. But as males approach spawning, aggression to intraspecific females changes to

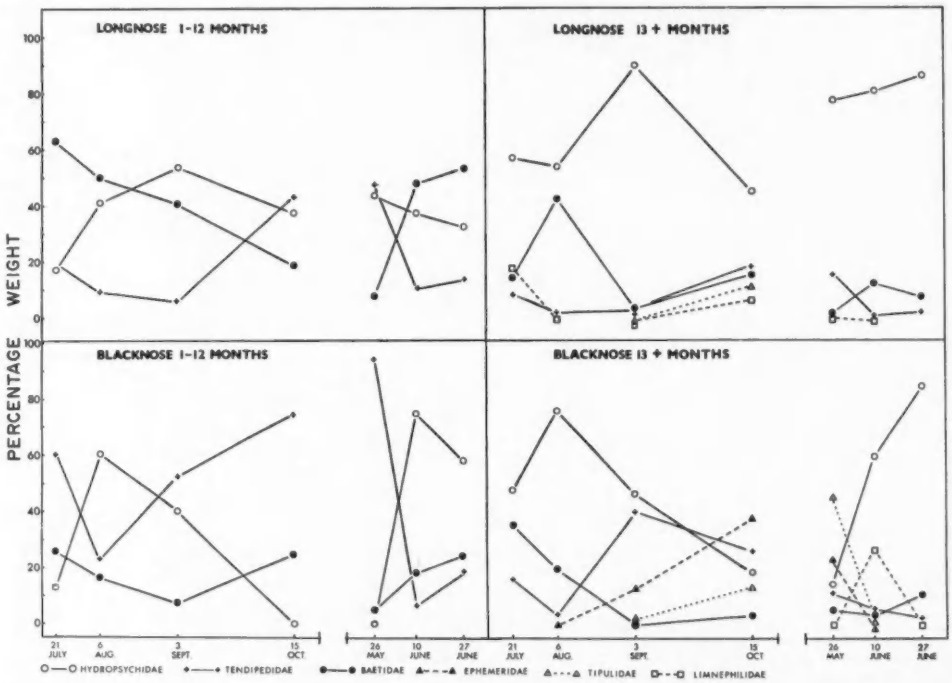


FIG. 3. Percentage weight of individual insect families consumed. Winter is represented by a break between October and May.

courtship (Bartnik 1970). In this situation, then, it is suggested that different environments are occupied by the sexes of each species to reduce intraspecific conflict between the sexes prior to spawning. (V. G. Bartnik personal communication).

In contrast to their spatial distribution, diet of the two species was, at times, strikingly similar both in quality and quantity. Divergences in the diet of older fishes, which occurred in September, October, and May, were almost certainly the result of blacknose having moved into slower, deeper waters, such as those of beaver ponds, during these months. Ephemeridae were abundant in this environment and became a common food item of blacknose at these times. Longnose remained in riffles and Hydropsychidae was maintained as the most important food item. Nevertheless, during the summer months, when most growth takes place, Hydropsychidae, followed by Baetidae and Tendipedidae, were the most important food items for both species.

The occurrence of spatially segregated sympatric species having a similar diet is unusual in freshwater fishes. Differences in diet are usually comparable with the degree of spatial segregation (Svardson 1949; Bernard MS 1972; Lindstrom and Nilsson 1962; Gee and Northcote 1963; Andrusak and Northcote 1971). Available food may be in short supply for both species of *Rhinichthys*, but, owing to spatial segregation, different portions of the same food crop are utilized. It has been noted that some spatial overlap does occur, but even in this situation different segments of the food supply may still be consumed, since longnose feed mainly between and under rocks and stones while blacknose forage on upper and dowstream surfaces (Gibbons MS 1971).

The cause of such differences in spatial distribution, due either to selective or interactive segregation (Brian 1956) is often most difficult to determine. Interactive segregation may play an important role in this study. For such a cause to operate, one or both species must exhibit considerable plasticity in their ecological requirements. This would allow for a shift in the preferred or optimal environment selected in the presence or absence of an interacting species (Nilsson 1967). Longnose and blacknose dace from the Mink River

can adjust their swimbladder volume in response to changes in water velocity. This would allow both species to occupy a variety of environments in time and space (Gee 1968, 1970). In addition, recent experimental evidence (Gee unpublished data) indicates that the growth of the swimbladder of longnose dace is influenced by the velocity of water in which the fish lives. In still water, growth is greatest, allowing individuals to reach a more buoyant condition than those reared in current. Thus, this species appears to be extremely plastic in terms of buoyancy, and this is reflected in its successful occupancy of a wide range of environments, from rapids in streams to the relatively still waters of lakes. Blacknose appear to be much more restricted in the range of environments occupied, living primarily in still-to-moderate currents of streams. If interactive segregation occurs in the Mink River, then blacknose must have some advantage over longnose dace in waters of low velocity, resulting in the displacement of the latter species from a portion of the potential range of environments it can occupy.

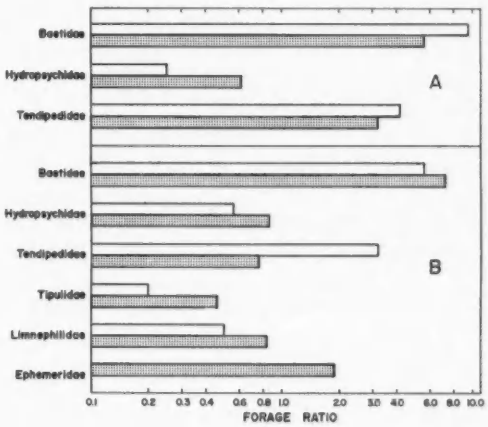


FIG. 4. Forage ratios for insect families consumed by longnose (open) and blacknose (stippled). Age 0 years (A) and 1+ years (B).

The mechanisms by which interactive segregation can operate are varied (Nilsson 1967), but exploitation, in which one species is able to utilize the environment under contest more efficiently than the other, is a distinct possibility. Neither species is territorial except during spawning.

Interactive and selective segregation appear to operate at different stages in the life cycle of longnose and blacknose dace. Selective segregation appears to be the case in the utilization of resources for spawning. Each species has evolved a complex set of behavior patterns that are adaptive for successful spawning in very different and restricted environments. The evolution of this behavior would be advantageous when a wide range of environments are present each spring. But interactive segregation appears to be the case for juveniles and older dace outside the reproductive period when selective segregation for a specific sort of environment would be of limited value in a stream, where conditions can change greatly over the year due to extreme variation in volume of water flowing.

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TABLE 2. Coefficients of percentage similarity of diet (LN, longnose dace; BN, blacknose dace; 1, age 0 years; 2, age 1+ years).

| | Type of comparison | | | |
|---|---|---|---|---|
| | Interspecific | | Intraspecific | |
| Date | $BN_1$ vs. $LN_1$ | $BN_2$ vs. $LN_2$ | $BN_1$ vs. $BN_2$ | $LN_1$ vs. $LN_2$ |
| July 21 | 59 | 72 | 55 | 41 |
| Aug. 6 | 67 | 77 | 74 | 87 |
| Sept. 3 | 53 | 50 | 81 | 61 |
| Oct. 15 | 63 | 42 | 30 | 73 |
| May 26 | 53 | 31 | 17 | 63 |
| June 10 | 63 | 68 | 69 | 53 |
| June 27 | 71 | 97 | 72 | 44 |

tional Research Council of Canada and the Fisheries Research Board of Canada.

ANDRUSAK, H., AND T. G. NORTHCOTE. 1971. Segregation between adult cutthroat trout (*Salmo clarki*) and Dolly Varden (*Salvelinus malma*) in small coastal British Columbia lakes. J. Fish. Res. Bd. Canada 28: 1259–1268.

BARTNIK, V. G. 1970. Reproductive isolation between two sympatric dace, *Rhinichthys atratulus* and *Rhinichthys cataractae*, in Manitoba. J. Fish. Res. Bd. Canada 27: 2125–2141.

BERNARD, D. J. MS 1972. Ecological divergence between emerald and spottail shiners (*Notropis*) in Lake Manitoba. M.Sc. Thesis. Univ. Manitoba, Winnipeg, Man. 97 p.

BRIAN, M. V. 1956. Segregation of species of the ant genus *Myrmica*. J. Anim. Ecol. 25: 319–337.

GAUSE, G. F. 1934. The struggle for existence. Williams and Wilkins Co., Baltimore, Md. 163 p.

GEE, J. H. 1968. Adjustment of buoyancy by longnose dace (*Rhinichthys cataractae*) in relation to water velocity. J. Fish. Res. Bd. Canada 25: 1485–1496.

1970. Adjustment of buoyancy in blacknose dace, *Rhinichthys atratulus*. J. Fish. Res. Bd. Canada 27: 1855–1859.

1972. adaptive variation in swimbladder length and volume in dace, genus *Rhinichthys*. J. Fish. Res. Bd. Canada 29: 119–127.

GEE, J. H., AND T. G. NORTHCOTE. 1963. Comparative ecology of two sympatric species of dace (*Rhinichthys*) in the Fraser River system, British Columbia. J. Fish. Res. Bd. Canada 20: 105–118.

GIBBONS, J. R. H. MS 1971. Comparative ecology of two sympatric species of dace *Rhinichthys cataractae* and *Rhinichthys atratulus*, in the Mink River, Manitoba. M.Sc. Thesis. Univ. Manitoba, Winnipeg, Man. 67 p.

HUBBS, C. L., AND K. F. LAGLER. 1964. Fishes of the Great Lakes region. The University of Michigan Press, Ann Arbor, Mich.213 p.

JONES, F. R. H. 1957. The swimbladder, p. 305–322. *In* M. E. Brown [ed.] The physiology of fishes. Vol. II. Academic Press, Inc., New York, N.Y.

LINDSTROM, T., AND N.-A. NILSSON. 1962. On the competition between whitefish species, p. 326–340. *In* E. D. Le Cren and M. W. Holdgate [ed.] The exploitation of natural animal populations. Blackwell Scientific Publications, Oxford, England.

MILLER, R. S. 1967. Patterns and processes in competition. Advan. Ecol. Res. 4: 1–74.

NELSON, E. M. 1961. The comparative morphology of the definitive swim bladder in the Catostomidae. Amer. Midland Natur. 65: 101–110.

NILSSON, N.-A. 1967. Interactive segregation between fish species, p. 295–313. *In* S. D. Gerking [ed.] The biological basis of freshwater fish production. Blackwell Scientific Publications, Oxford, England.

SVARDSON, G. 1949. Competition between trout and char (*Salmo trutta* and *Salvelinus alpinus*). Rep. Inst. Freshwater Res. Drottningholm, 29: 108–111.

USINGER, R. L. 1956. Aquatic insects of California. University of California Press, Berkeley and Los Angeles, Calif. 508 p.

WHITTAKER, R. H., AND C. W. FAIRBANKS. 1958. A study of plankton copepod communities in the Columbia Basin, southeastern Washington. Ecology 39: 46–65.

# Diel Periodicity of Chlorophyll *a* Concentration in Oregon Coastal Waters[1]

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GLOOSCHENKO, W. A., H. CURL JR., AND L. F. SMALL. 1972. Diel periodicity of chlorophyll *a* concentration in Oregon coastal waters. J. Fish. Res. Bd. Canada 29: 1253–1259.

Concentrations of chlorophyll *a* in phytoplankton exhibited a diel periodicity in Oregon coastal waters. Maximum surface concentrations often occurred around midnight and highest 25-m concentrations early in the evening (or even in late afternoon). Concentrations at intermediate depths fell in between and in a predictable progression with depth and time of day. Minimum chlorophyll *a* values occurred in the afternoon. No definite periodicity was established at 50 m.

Laboratory studies with *Skeletonema costatum* demonstrated that the diel cycle of chlorophyll *a* per cell was related to the light intensity and duration to which cells were exposed. Highest concentrations of this pigment occurred early in the dark period and lowest concentrations in the light period when cells were grown under photoperiods of 9, 12, and 15 hr at a light intensity of approximately 1200 ft-c. Pigment bleaching probably was responsible for the low concentrations during the light period. The decline of chlorophyll *a* from the maximum early in the dark period began after the cells possibly became deficient in some chlorophyll precursor or energy-yielding substrate. Addition of an external carbon source during the dark period prolonged the high chlorophyll *a* concentrations in the dark before the decline began. Under low light (400 ft-c) chlorophyll *a* synthesis occurred only in the light. This phenomenon was most likely due to an insufficient amount of energy-yielding substrate or precursor synthesized during the low-light period, and the lack of bleaching in the light period at this lower intensity. The laboratory results were consistent with interpretations of the field data. A correction for diel pigment periodicity is recommended for models estimating photosynthesis from chlorophyll and light data and for oceanographic surveys during which sampling of chlorophyll *a* is carried out throughout the 24-hr day.

GLOOSCHENKO, W. A., H. CURL JR., AND L. F. SMALL. 1972. Diel periodicity of chlorophyll *a* concentration in Oregon coastal waters. J. Fish. Res. Bd. Canada 29: 1253–1259.

La teneur en chlorophylle *a* du phytoplancton des eaux côtières de l'Orégon montre une périodicité nycthémérale. Les concentrations de surface atteignent souvent un maximum vers les minuit, alors que celles de 25 mètres en atteignent un tôt dans la soirée (ou même en fin d'après-midi). Aux profondeurs intermédiaires, les valeurs se situent entre les deux et suivent une progression prévisible liée à la profondeur et à l'heure du jour. Le minimum de chlorophylle *a* se produit dans l'après-midi. Il n'y a pas de périodicité bien définie à 50 mètres.

Des études au laboratoire sur *Skeletonema costatum* démontrent une relation entre le cycle nycthéméral de la chlorophylle *a* dans la cellule et l'intensité et la durée d'éclairage auxquelles les cellules sont exposées. Quand celles-ci sont cultivées à des photopériodes de 9, 12 et 15 heures, à une intensité lumineuse d'environ 1200 pieds-bougies, le maximum de concentration du pigment a lieu au début de la période d'obscurité, et le minimum durant la période d'éclairage. Le blanchiment du pigment est probablement responsable des faibles concentrations durant la période d'éclairage. Le déclin de la chlorophylle *a* qui suit le maximum du début de la période d'obscurité se fait sentir possiblement après l'épuisement de quelque précurseur chlorophyllien ou substrat énergétique dans la cellule. L'addition d'une source externe de carbone durant la période d'obscurité prolonge la durée du maximum et retarde le début du déclin. A une faible intensité lumineuse (400 pieds-bougies), la synthèse de la chlorophylle *a* ne se produit que durant la période d'éclairage. Ce phénomène est sans doute dû à l'insuffi-

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sance de la quantité de substrat énergétique ou de précurseur synthétisé et à l'absence de blanchiment durant la période d'éclairage à une intensité lumineuse diminuée. Les résultats de laboratoire sont en accord avec les données recueillies sur le terrain. Les modèles servant à estimer la photosynthèse à partir de données sur la chlorophylle et la lumière, ainsi que les relevés océanographiques avec échantillonnage de chlorophylle *a* sur périodes de 24 heures devront tenir compte de la périodicité nycthémérale du pigment et être corrigés en conséquence.

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THIS study was performed to investigate factors, especially light, influencing diel changes in the marine phytoplankton standing crop as measured by chlorophyll *a*. Previous diel pigment studies in the oceans have been conflicting. For example, Yentsch and Scagel (1958), Steeman-Nielsen and Jorgensen (1962), El-Sayed and Mandelli (1965), and Glooschenko (1970) found that highest in situ chlorophyll values occurred at night. Highest chlorophyll values during the day, and lowest values at night were reported by Shimada (1958), Yentsch and Ryther (1957), Ryther et al. (1961), Lorenzen (1963), McAllister (1963), and Wood and Corcoran (1966).

In order to explain these discrepancies and to investigate the causes of diel pigment periodicity, field and laboratory experiments were performed with chlorophyll *a*: in mixed assemblages of phytoplankton in the field and with *Skeletonema costatum* in culture. These field studies differed from the previously mentioned studies in their distribution over different times of the year. In the culture studies, light intensity and photoperiod were varied and chlorophyll *a* was measured on a per cell basis.

## Methods

### SAMPLING PROCEDURE AT SEA

Samples were taken in Oregon coastal waters and at two mid-Pacific stations, one north of Hawaii and the other south of the Aleutian Islands. All sampling was done aboard the Oregon State University research vessel R/V Yaquina. Upon arriving on station, the ship was allowed to drift in order to sample approximately the same population of phytoplankton throughout a 24-hr period.

Water samples were collected approximately every 2 hr, while on station, with Van Dorn bottles from the surface and depths of 10, 15, 25, and 50 m. Phytoplankton was filtered through 0.8- $\mu$ membrane filters which were stored in a desiccator kept in the ship's freezer. Pigment analyses were performed as described by Strickland and Parsons (1965) except that samples were extracted in 90% acetone for 1 min using a Sorvall high speed homogenizer. Preliminary tests had shown that all pigments were extracted by this procedure. Solar radiation was measured by means of a pyrheliometer on the mid-Pacific stations.

### LABORATORY EXPERIMENTS

*Skeletonema costatum* Cleve isolated from Oregon coastal water was used for all laboratory studies. Cultures were not bacteria-free. The medium used to grow this species was prepared by autoclaving 0.45-$\mu$ membrane-filtered seawater and enriching it with various nutrients (Curl 1962), including the addition of 1 gm/liter thiourea.

In most of the experiments, use was made of a Sherer–Gillet® growth chamber (Model CEL 34–7) programmed for light intensity and photoperiod by switching three sets of incandescent and fluorescent lights. A five-compartment box was placed inside the growth chamber. Each compartment was covered by a Corning® molded, unpolished glass filter chosen to simulate the intensity and quality of light at 10 and 50 m depth in the ocean (Table 1). The lights were turned on and off by timers to simulate sunrise, morning, noon, afternoon, and sunset. Each compartment held one 3.8 liter polyethylene collapsible water bottle. All experiments were performed at 18 C to duplicate temperature conditions in the culture room.

For one experiment requiring the measurement of pigments every 2 hr, 12 liters of enriched seawater were prepared and inoculated with 100 ml of *S. costatum* culture. The light intensity during light periods was 400 ft-c from "deluxe warm-white" fluorescent lamps. During dark periods, the culture vessel was covered with a hood. Temperature was kept at 18 $\pm$ 1 C. Filtered air was bubbled into the culture to keep it well mixed. Every 2 hr, samples were drawn from the culture for pigment analyses and cell counts. The pigment analyses were performed in the same way as described for the samples collected at sea. Cell counts were made with a Fuchs-Rosenthal counting chamber.

In addition to experiments performed to study the influence of photoperiod and light intensity on diel

TABLE 1. Light types, energy, and filters used in laboratory culture experiments.

| Simulated Depth (*m*) | Corning Filter No. | Radiant energy Langleys per min | | |
|---|---|---|---|---|
| | | Low Light[a] | Medium[b] | High[c] |
| "0" | none | 0.001 | 0.041 | 0.050 |
| 10 | 2781 | 0.0003 | 0.010 | 0.015 |
| 50 | 2827 | 0.0001 | 0.003 | 0.004 |

[a]From four 60-watt incandescent lamps.
[b]From six Sylvania 48" daylight, type F48T12-GRO-VHO, and four 60-watt incandescents.
[c]From two Sylvania Grow-Lux, type F48T12-GRO-VHO, four Sylvania daylight, type F48T12-D-VHO, and one GE photoreproduction, type F48PG 17.1PR in addition to lights in medium light treatment.

chlorophyll *a* changes, two other sets of experiments were performed with cultures. One set was designed to measure instantaneous effect of high light intensity on chlorophyll *a* bleaching. A 3-liter culture of *S. costatum* grown to log phase (210, 000 cells/ml) at a light intensity of 200 ft-c was placed in the dark for 24 hr. Fifty-ml aliquots were placed in a filter funnel in the dark and then exposed to light at 6500 ft-c from a quartz-iodine lamp for 5, 30, 60, and 180 sec. Cells were filtered immediately and analyzed for chlorophyll *a*. Cell counts were made on each sample.

## Periodicities in Field

Diel periodicity of chlorophyll *a* concentrations occurred in the coastal waters in the summer, autumn, and winter, even though concentrations of chlorophyll were considerably different in three seasons (Fig. 1). The timing of chlorophyll highs and lows varied somewhat by season and depth. However, features that appeared consistently were (1) occurrence of peak concentrations at night or early morning, and (2) a phase shift in which peaks occurred earlier in the night with increasing water depths sampled. There was a suggestion of two night-time peaks in the January 1967 data with a general decline at about 0100 hr (Fig. 1a). Similar results were obtained for observations on July 20–21, 1965 (Fig. 1b), September 25–26, 1965, (Fig. 1c), and November 28–30, 1965 (not shown), although the chlorophyll maxima during the last period were not as distinct as those in January, July, and September possibly due to turbulent mixing caused by a storm that occurred during the November cruise. At the station north of Hawaii (Fig. 1d) and at 50° 28′N, 176° 14′W (not shown) in June 1966, the periodicity patterns were similar to that near the Oregon coast.

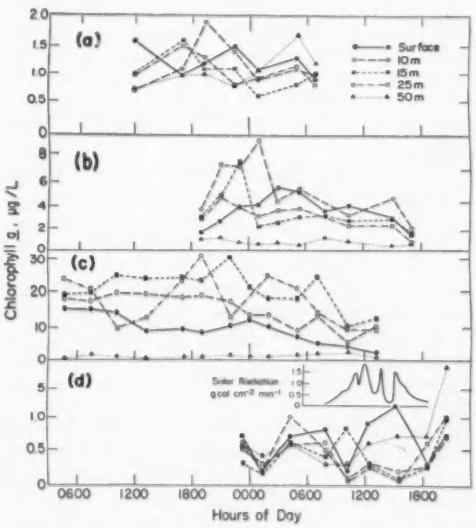


FIG. 1 In situ chlorophyll *a* concentrations during daily cycles (a) January 3–4, 1967 at 44° 39′N, 124° 39′W, (b) July 20–21, 1965 at 44° 39′N, 124°39′W, (c) September 25–26, 1965 at 44°21′N, 124°29′W, (d) June 9, 1966 in mid-Pacific Ocean at 29°12′N, 161°30′W.

Data from 0800 to 2100 at 29° 12′N, 161° 30′W on June 9, 1966, indicate a relation between chlorophyll *a* concentration and solar radiation (Fig. 1d). The day of June 9 was characterized by bright sun and cloudless skies except during a period from about 1200 to 1500 hr when a dense cloud cover was present. Surface chlorophyll *a* concentrations increased markedly during the lowered radiation levels at mid-day while concentrations at depths below the surface remained largely unchanged. The increase in concentrations at all depths at 2100 hr over those at 1830 hr should have been the beginning of the expected night-time peak. On no other generally sunny day of sampling was the solar radiation level so low for so long during the middle of the day.

## Periodicities in Laboratory

In the first laboratory experiment (Fig. 2a), *S. costatum* was exposed to 12-hr periods of light and dark at a light intensity (400 ft-c) comparable to solar radiation at 25 or 30 m depths over the Oregon continental shelf on a sunny day. Chlorophyll *a* concentration increased in the light period to maximum value at the end of the light period or early dark period. It then decreased during the following dark period.

When *S. costatum* was exposed to 12-hr periods of light and dark at simulated surface and 10 and 50 m depths (Fig. 2b) chlorophyll *a* at the "surface" and "10 m" generally showed maximum concentrations during the 12-hr dark periods and minimum concentrations in the 12-hr light periods. This was in contrast to the increase of pigment at the end of the light period and decrease in the dark as seen in Fig. 2a. At the "50 m" depth no periodicity was observed.

In another experiment, a 16-hr light–8-hr dark cycle (Fig. 2c) was used to simulate a summer light-dark sequence. At the "surface," cholrophyll *a* values in the dark periods tended to be higher than those in the light periods. At "10m" and "50 m," however, peaks were attained from the middle of the light period to the beginning of the dark period, with minima in dark to early-light periods.

In a 9-hr light–15-hr dark experiment simulating winter conditions (not shown) chlorophyll *a* per

cell generally showed a diel periodicity at all three "depths." Maximum values occurred at the end of the light periods or early in the dark periods, and minimum values at the end of the dark periods or early in the light periods.

In a preliminary bleaching experiment, the concentration of chlorophyll *a* in cells previously held in the dark for 24 hr increased 22% with a 5-sec exposure to 6500 ft-c and 30% in 30 sec (Fig. 3). The concentration declined with longer exposure to high light intensity, so that at 180 sec it was only 57% of the concentration in the dark, which was a stable 137 µg Chl *a* per liter throughout the 180-sec experiment. When a culture was kept under 200 ft-c rather than being placed in the dark for 24 hr, chlorophyll *a* remained practically constant up to 30 sec but declined to 62% of that at 200 ft-c after a 180-sec exposure to 6500 ft-c. The concentration at 200 ft-c remained at 166 µg Chl *a* per liter. In a third experiment, cells previously kept under 200 ft-c were exposed in one case to 3800 ft-c from the tungsten–iodide lamp and in another case to 2100 ft-c. After 180 sec at 3800 ft-c chlorophyll *a* concentration dropped to 89% of the control, but after the same length of time at 2100 ft-c there was no bleaching.

An experiment was also performed to study the effect of supplying an organic carbon source to the cells during the period of decline of chlorophyll in the dark. In the culture to which the external carbon source was added, the concentration of chlorophyll *a* per cell increased in the light and stayed relatively high in the dark for at least 4 hr (Fig. 4). Chlorophyll *a* concentration in a control culture, with no added carbon, experienced a general decline through the dark period.

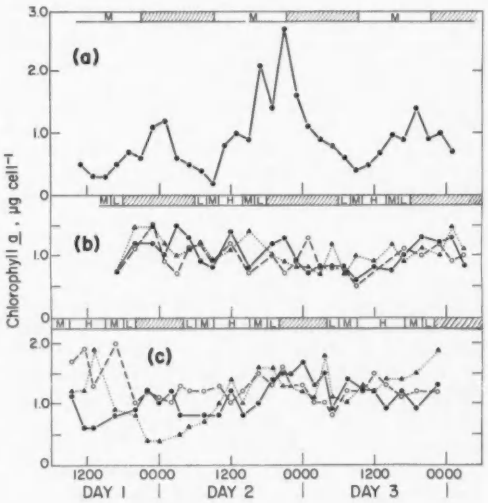


FIG. 2 Chlorophyll *a* per cell in *Skeletonema costatum* under (a) 12-hr photoperiod, 400 ft-c (b) 12-hr photoperiod, variable intensity, and (c) 16-hr photoperiod, variable intensity. Hatched bar indicates dark, L, low light intensity (Table 1); M, medium; H, high. ●, "surface"; ○, "10 m"; ▲, "50 m."

## Discussion

The laboratory experiments indicate that light intensity and length of photoperiod affect the diel periodicity of pigments, as predicted by Terborgh and Thimann (1964). At low light intensities, or with short light duration, chlorophyll *a* increases in the light (Fig. 2). These results generally agree with previous work done on other algal species (Gibor and Meehan 1961; and Eppley and Coatsworth 1966). Unfortunately, these authors expressed their results as chlorophyll per volume of culture solution In a dividing population of cells, results expressed as chlorophyll per cell are more meaningful. Species differences may also influence time of chlorophyll synthesis; Paasche (1967) found *Coccolithus huxleyi* to form chlorophyll *a* in both the light and dark, while, primarily under similar conditions, he (Paasche 1968) found two diatom species to synthesize chlorophyll *a* in the light. Jorgensen (1966) concluded that synchronous cell division during the light period was the cause of diel pigment periodicity. Synchronous cell division during either the dark or light period was not observed in any of our experiments.

At the higher light intensities used in our experiments, i.e. at the intensities used at the "surface" and "10 m depth" (Fig. 2b, c), chlorophyll *a* was at its lowest concentration during part of the light period, and increased to a maximum during the dark period as a result of some pigment bleaching in the higher light intensities. Yentsch and Scagel (1958) attributed the decline in chlorophyll concentration in high light to photo-oxidation of pigment. Sironval and Kandler (1958) demonstrated with *Chlorella* grown at high light intensities that bleaching of the chlorophyll molecule occurred only at light intensities greater than 4650 ft-c, however, with a seven-fold increase in bleaching occurring between 6500 and 9300 ft-c. These intensities were substantially higher than those employed in the development of our "surface" and "10 m" curves in Fig. 2, but this fact does not preclude the possibility that our *S. costatum* cultures were adapted to low light and began to bleach with even a modest increase in light intensity. Bleaching

is rapidly initiated under high light (Fig. 3). Also significant is the fact that cells held in the light apparently are more "resistant" to pigment bleaching than those kept in darkness for 24 hr which suggests that light history of the cells is an important factor, as postulated by Yentsch and Scagel (1958) in determining the degree of diel chlorophyll periodicity.

Decline of chlorophyll *a* in the dark after a history of low illumination or short photoperiod may be the result of a decline in formation of a chlorophyll precursor or energy-yielding substrate. In our experiments, addition of an external carbon source during the dark period maintained chlorophyll *a* concentration per cell at a level higher than a control without added substrate (Fig. 4). Frank and Kenney (1955) demonstrated a similar phenomenon with corn seedlings. In their experiments, additions of 3% sucrose solution delayed chlorophyll depletion in the dark and prevented destruction of the chlorophyll molecule after 120 hr of darkness. Wolff and Price (1960) found stimulation of chlorophyll biosynthesis in higher plant leaves with sugar additions.

Increases in chlorophyll *a* per cell in the dark, noted after growth in relatively high light and long photoperiod, probably were the result of an adequate amount of chlorophyll precursor or organic substrate synthesized during the light period. Bogorad (1965) reported that several species of algae can synthesize chlorophyll in the dark and therefore must have enzyme systems capable of reducing either protochlorophyll or protochlorophyllide *a* in the dark. Castenholz (1964) and Yentsch (1965) found chlorophyll *a* synthesis in the dark by marine diatoms. Protein content may be the limiting factor for chlorophyll synthesis (Nadler and Granick 1970).

Generalized curves of chlorophyll *a* periodicity under both low and high light conditions derived from our experimental results (Fig. 5) are compatible with the results of others. The apparent discrepancies in the literature are resolved by examining the available pre-history of light intensity and photoperiod for each observation. For example, increases in chlorophyll in the light period reported by Gibor and Meehan (1961), Terborgh and Thimann (1964), Edmonds (1965), Eppley and Coatsworth (1966) and Jorgensen (1966) were found under experimental light intensities ranging form 250 to 300 ft-c. Increases in chlorophyll in the dark reported by Yentsch and Reichert (1963), Castenholz (1964) and Yentsch (1965), were found when light intensities between about 800 and 1500 ft-c were used to grow the cultures. Beale and Appleman (1971) demonstrated that in *Chlorella*, chlorophyll is present at high concentrations when light is limiting for growth and in low concentrations when light is abundant. Our results were quite similar.

Though it has been postulated that the chlorophyll minimum at night or during the early morning could be produced as a result of heavy zooplankton grazing (McAllister 1963; Wood and Corcoran 1966), off the Oregon coast the maximum chlorophyll

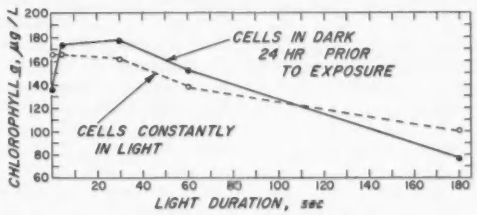


FIG. 3. Chlorophyll *a* bleaching under exposure to 6500 ft-c in relation to previous light history of cells.

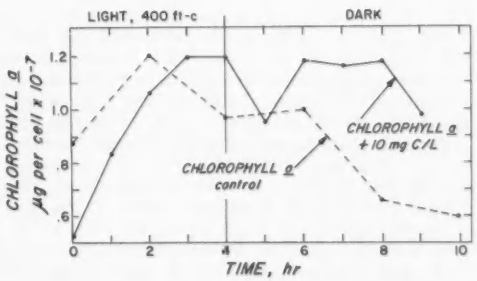


FIG. 4 Effect of external carbon source (5 mg/liter glucose + 5 mg/liter pyruvate) on chlorophyll *a* concentrations in *Skeletonema costatum*.

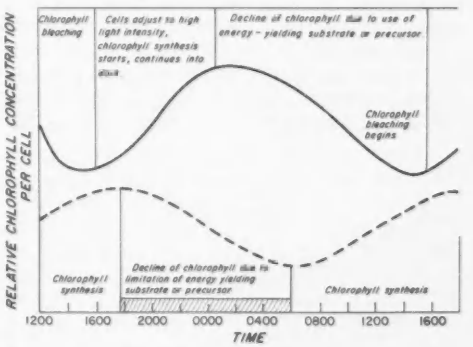


FIG. 5 Summary of diel chlorophyll *a* fluctuations in *Skeletonema costatum* under a 12-hr photoperiod at high (*top curve*) and low (*bottom curve*) light intensities. Hatched bar, dark.

concentrations occur at night, in the face of intensified grazing pressure by herbivores. We have echo-sounder traces of the deep scattering layer (DSL) taken off Oregon since February 1966, and in general the layer is below 150 m during the daylight hours, begins to ascend at sunset, reaches to within 15–20 m of the surface by about 1900 hr, and remains at this level until sunrise. The DSL is not composed entirely of herbivores but any contribution of grazers to the surface layers by the DSL represents an increase over daytime concentrations. Also, cell division rates would have to be much higher than normally occur in nature to explain the magnitude of chlorophyll *a* increases during the day and early evening (Glooschenko 1970).

One result of diel periodicity of chlorophyll *a* in marine phytoplankton populations appears to be that of damping extreme oscillations in production. In low light, chlorophyll is synthesized and assimilation of light energy is enhanced. In more intense light, bleaching decreases chlorophyll concentration, which might prevent the manufacture of substances harmful to the cell (Sironval and Kandler 1958; Kandler and Sironval 1959). The concept of chlorophyll *a* as a governor on photosynthetic rate is not new and plays a part in the concept of "sun" and "shade" species of phytoplankton (Ryther and Menzel 1959; Steemen-Nielsen and Hansen 1959; Yentsch and Lee 1966) as well as in diel photosynthetic periodicity.

One of the first models proposed for the estimation of daily primary production from chlorophyll and light data (Ryther and Yentsch 1957) required only one depth series of chlorophyll *a* measurements during the day. The treatment of the chlorophyll components of the model as a variable rather than a constant would enhance the precision and usefulness of the model. One way to introduce this variable is to develop a correction curve so that chlorophyll *a* values obtained at any time during a 24-hr period can be corrected to a standard time of day. In Fig. 6 we give correction curves for five depths to convert chlorophyll *a* estimates at any time of day or night to a standard time of 1000 hr, PST. We selected 1000 hr because in Oregon coastal waters at that time photosynthesis is relatively high, surface bleaching of pigments is relatively low, and migratory grazing herbivores have descended from the surface layers. The curve also can be used to generate chlorophyll *a* concentrations at other times of day or night, once the concentrations at 1000 hr are established. Such a procedure would be useful in correcting chlorophyll *a* values collected on research cruises which collect samples on a 24-hr basis over wide areas.

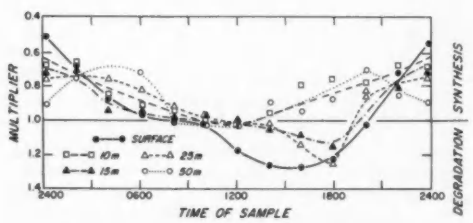


FIG. 6 Correction curve for in situ diel periodicity of chlorophyll *a* in Oregon coastal waters.

## Acknowledgments

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BEALE, S. I., AND D. APPLEMAN. 1971. Chlorophyll synthesis in *Chlorella*. Plant Physiol. 47: 230–235.

BOGORAD, L. 1965. Chlorophyll biosynthesis, p. 29–74. *In* T. W. Goodwin [ed.] Chemistry and biochemistry of plant pigments. Academic Press Inc., New York, N.Y.

CASTENHOLZ, R. W. 1964. The effect of daylength and light intensity on the growth of littoral marine diatoms in culture. Physiol. Plant. 17: 951–963.

CURL, H. C., Jr. 1962. Effect of divalent sulfur and vitamin $B_{12}$ in controlling the distribution of *Skeletonema costatum*. Limnol. Oceanogr. 7: 422–424.

EDMONDS, L. N. 1965. Studies on the synchronously dividing cultures of *Euglena gracilis* Klebs (strain Z). II. Pattern of biosynthesis during the cell cycle. Cell. Comp. Physiol. 66: 159–181.

EL-SAYED, S. Z., AND E. F. MANDELLI. 1965. Primary production and standing crop of phytoplankton in the Weddell Sea and Drake Passage, p. 87–106. *In* G. A. Lalno [ed.] Biology of the Antarctic Seas. Vol. 2. American Geophysics Union Publication 1297, Washington, D.C.

EPPLEY, R. W., AND J. L. COATSWORTH. 1966. Culture of the marine phytoplankter *Dunaliella tertiolecta*, with light-dark cycles. Arch. Mikrobiol. 55: 66–80.

FRANK, S., AND A. KENNEY. 1955. Chlorophyll and carotenoid destruction in the absence of light in seedlings of *Zea Mays*. Plant. Physiol. 30: 413–418.

GIBOR, A., AND W. R. MEEHAN. 1961. Diurnal variations in chlorophyll *a* content of some freshwater algae. Ecology 42: 156–157.

GLOOSCHENKO, W. 1970. Diel periodicity of chlorophyll *a* in the Gulf of Mexico. Quart. J. Fla. Acad. Sci. 33: 187–192.

JORGENSEN, E. G. 1966. Photosynthetic activity during the life cycle of synchronous *Skeletonema* cells. Physiol. Plant. 19: 786–799.

KANDLER, O., AND C. SIRONVAL. 1959. Photo-oxidation processes in normal green *Chlorella* cells. Biochim. Biophys. Acta 33: 207–214.

LORENZEN, C. H. 1963. Diurnal variation in photosynthetic activity of natural phytoplankton populations. Limnol. Oceanogr. 8: 56–62.

MCALLISTER, C. D. 1963. Measurements of diurnal variation in productivity at ocean station "P". Limnol. Oceanogr. 8: 289–292.

NADLER, K., AND S. GRANICK. 1970. Controls on chlorophyll synthesis in barley. Plant Physiol. 46: 240–246.

PAASCHE, E. 1967. Marine plankton grown with light-dark cycles I. *Coccolithus huxleyi*. Physiol. Plant. 20: 946–956.

1968. Marine plankton grown with light dark cycles II. *Nitzschia turgidula*. Physiol. Plant. 21: 66–77.

RYTHER, J. H., AND D. W. MENZEL. 1959. Light adaptation by marine phytoplankton. Limnol. Oceanogr. 4: 492–497.

RYTHER, J. H., D. W. MENZEL, AND R. F. VACCARO. 1961. Diurnal variations in some chemical and biological properties of the Sargasso Sea. Limnol. Oceanogr. 6: 149–153.

RYTHER, J. H., AND C. S. YENTSCH. 1957. The estimation of phytoplankton production in the ocean from chlorophyll and light data. Limnol. Oceanogr. 2: 281–286.

SHIMADA, B. M. 1958. Diurnal fluctuation in photosynthetic rate and chlorophyll *a* content of phytoplankton from Eastern Pacific waters. Limnol. Oceanogr 3: 336–339.

SIRONVAL, C., AND O. KANDLER. 1958. Photo-oxidation process in normal green *Chlorella* cells. I. The bleaching process. Biochim. Biophys. Acta 29: 359–368.

STEEMAN-NIELSEN, E., AND V. K. HANSEN. 1959. Light adaptation in marine phytoplankton populations and its interrelationship with temperature. Physiol. Plant. 12: 353–370.

STEEMAN-NIELSEN, E., AND E. G. JORGENSEN. 1962. The physiological background for using chlorophyll measurements in hydrobiologie and a theory explaining daily variations in chlorophyll concentration. Arch. Hydrobiol. 58: 349–357.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1965. A manual of seawater analysis. Fish. Res. Board Can. Bull. 125: 203 p.

TERBORGH, J., AND K. V. THIMANN. 1964. Interactions between day length and light intensity in the growth and chlorophyll content of *Acetabularia crenulata*. Planta 63: 83–98.

WOLFF, J. B., AND L. PRICE. 1960. The effect of sugars on chlorophyll biosynthesis in higher plants. J. Biol. Chem. 235: 1603–1608.

WOOD, E. J. F., AND E. F. CORCORAN. 1966. Diurnal variation in phytoplankton. Bull. Mar. Sci. 16: 383–403.

YENTSCH, C. S. 1965. Distribution of chlorophyll and phaeophytin in the open ocean. Deep-Sea Res. Oceanogr. Abstr. 12: 653–666.

YENTSCH, C. S., AND R. W. LEE. 1966. A study of photosynthetic light reactions and a new interpretation of sun and shade phytoplankton. J. Mar. Res. 24: 319–337.

YENTSCH, C. S., AND C. A. REICHERT. 1963. The effects of prolonged darkness on photosynthesis, respiration, and chlorophyll in the marine flagellate, *Dunaliella euchlora*. Limnol. Oceanogr. 8: 388–342.

YENTSCH, C. S., AND J. R. RYTHER. 1957. Short-term variations in phytoplankton chlorophyll and their significance. Limnol. Oceanogr. 2: 140–142.

YENTSCH, C. S., AND R. F. SCAGEL. 1958. Diurnal study of phytoplankton pigments: an *in situ* study in East Sound, Washington. J. Mar. Res. 17: 567–583.

# Estimates of Primary Production off Oregon Using an Improved Chlorophyll-Light Technique[1]

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An existing equation for the estimation of primary production from chlorophyll and light data was examined, and revisions were made to allow more precise applications of the equation to upwelling areas and other regions with relatively large fluctuations in chlorophyll concentration and production per unit of chlorophyll during the day. The revised equation was developed by estimating production in 2-hr increments through the daylight period, and integrating by parts to arrive at production in terms of gC $m^{-2}$ $day^{-1}$. Total daily production in coastal waters was estimated within $\pm 13\%$ of mean $^{14}C$ estimates, on the average, while in offshore waters the estimates averaged within $\pm 21\%$ of mean $^{14}C$ estimates. Estimates by the revised equation averaged 52% better in coastal waters, and 32% better in offshore waters, than the basic, unrevised equation.

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Après examen de l'équation servant présentement à estimer la production primaire à partir de données sur la chlorophylle et la lumière, les auteurs y apportent des corrections permettant une application plus précise aux régions de remontée d'eaux profondes et aux régions où les fluctuations de la teneur en chlorophylle et de la production diurne par unité de chlorophylle sont relativement grandes. A cette fin, ils estiment la production par accroissements de deux heures durant la période diurne d'éclairage, et intègrent par parties pour en arriver à la production en termes de gC $m^{-2}$ $jour^{-1}$. Ils obtiennent ainsi un estimé de la production journalière totale qui, dans les eaux côtières, est en moyenne de $\pm$ 13% de l'estimé moyen du $^{14}C$, alors que, dans les eaux du large, la valeur correspondante est de $\pm$ 21%. L'équation revisée a fourni des estimés dépassant en précision ceux obtenus à l'aide de l'équation de base non revisée, dans une proportion de 52% dans les eaux côtières et de 32% dans les eaux du large.

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RYTHER (1956), Ryther and Yentsch (1957), and Talling (1957) have developed methods to predict daily primary production, based on photosynthetic responses to incident and penetrating light, or to incident light and measured chlorophyll concentration at a given time of day. Vollenweider (1965) has given a theoretical treatment of day rate production curves, with the change in incident light with time as the variable of interest. In the above analyses, account was not taken of changes in chlorophyll content in the water column with time of day, or changes in production per unit of chlorophyll with either time of day or changing nutrient concentrations on different days.

Glooschenko et al. (1972) have indicated that diel chlorophyll *a* periodicity might be a significant factor in estimating daily production, at least in coastal waters. Such periodicity is affected by light intensity; thus, on sunny days one might expect higher chlorophyll concentrations in early morning than at midday or in the afternoon, while on heavily overcast days one might expect chlorophyll concentrations to be low in the morning and to increase throughout the daylight period. In addition, nonperiodic response of phytoplankton

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chlorophyll to gross changes in light intensity during a single day can be pronounced, with a time scale of change in the order of minutes to hours. It is also known that both chlorophyll (Thomas 1970b) and production/chlorophyll (Curl and Small 1965; Thomas 1970a; Glooschenko and Curl 1971) are affected by nutrient availability in upper waters.

In the Oregon coastal region, and perhaps in regions of upwelling in general, rapid temporal changes in input of solar radiation are common. Often fog and/or clouds completely obscure the sun for the total daylight period, but just as common are days in which the sky cover is complete for segments of the day but clear sky prevails in the intervening times. Nutrient input into surface waters via upwelling off Oregon usually has a slower time rate of change than solar radiation input, but one must be cognizant of these changes because of the effect of nutrient availability on production/chlorophyll ratios. In this paper we have undertaken a reexamination of the Ryther and Yentsch (1957) equations, made some revisions which allow better application of the equations to upwelling areas, and tested the revised procedure against in situ $^{14}C$ measurements in the sea. If upwelling regions are responsible for a significant fraction of total oceanic production (Ryther 1969), then the development of equations to yield more precise productivity estimates in such regions should be valuable.

## Methods

The sampling and analytical procedures for estimation of chlorophyll *a* concentrations are given in Glooschenko et al. (1972). In situ $^{14}C$ experiments were performed as follows: Water with its endemic phytoplankton was collected with large-volume, nontoxic sampling bottles from the surface and from depths of 10, 25, 50, and 100 m approximately every 2 hr from sunrise to sunset. On several occasions the samples were taken at the surface and at 10, 15, 25, and 50 m. Subsamples from each depth were placed into two clear glass bottles and one darkened glass bottle, and each subsample was inoculated with 4$\mu$Ci $NaH^{14}CO_3$. The glass bottles were then resuspended at the depth from which the water was originally collected. Net production was determined by the method of Steeman-Nielson (1952), but with a modification of the $^{14}C$ standardization procedure (Small 1967). Production values, initially expressed as gC $m^{-3}$ $hr^{-1}$, were integrated over depth and daylength to yield production in terms of gC $m^{-2}$ $day^{-1}$. A minimum production estimate for each day was generated by using the subsamples giving the lowest productivity for each 2-hr period of the day, and a maximum daily estimate was calculated from the subsamples giving the highest productivity for each 2-hr period. The difference between minimum and maximum daily estimates was itself an estimate of the range of variability of the $^{14}C$ experiment for the particular day. Mean daily $^{14}C$ production values, plus or minus the half-ranges of variability associated with each mean production estimate, are given in Table 1, column A.

Surface radiation was monitored continually in gcal $cm^{-2}$ $day^{-1}$ with a pyranometer, and submarine radiation was measured concurrently with the $^{14}C$ experiments at sea to establish the photic depth. An underwater barrier cell irradiance meter with cosine collector was used for the submarine radiation measurements. In addition, sky cover data for our sampling area and dates were available from the ESSA weather station in Astoria, Oreg., and such data indicated that our sampling days ranged from completely open sky conditions to completely overcast conditions (Table 1)

## The Basic Equation

The most useful equation developed by Ryther and Yentsch (1957), applicable under conditions of nonhomogeneous distribution of chlorophyll *a* in the photic zone, is:

$$P_d = R_d \times C_d \times 3.7$$

where $P_d$ = photosynthesis during the daylight day (gC $m^{-3}$ $day^{-1}$) at depth $d$ in the photic zone, $R_d$ = relative photosynthesis (R $m^{-3}$ $day^{-1}$) at $d$ as a function of total daily surface radiation, $C_d$ = measured chlorophyll *a* concentration (g Chl *a* $m^{-3}$) at one time of day at $d$, and 3.7 is an empirical assimilation number [gC $hr^{-1}$ (g Chl *a*)$^{-1}$ at light saturation]. $P_d$ can be calculated at depths corresponding to 100, 50, 25, 10, and 1% of the light incident to the surface, and plotted against $d$. Integration of the resulting curve yields an estimate of total production beneath a square meter of sea surface during the daylight day (P = gC $m^{-2}$ $day^{-1}$).

$R_d$ values are obtained from $R_d$ curves given as Fig. 1 in Ryther and Yentsch (1957). These curves were developed from three basic relations: (1) mean relative photosynthesis as a function of light intensity (P:I curve), (2) light intensity at the sea surface as a function of time of day (I:T curves), and (3) mean relative photosynthesis as a function of time of day (P:T curves) (Ryther 1956). The P:I curve was derived from experiments with representative marine phytoplankton species. Expression of photosynthesis in relative terms (the ratio of photosynthesis at a given intensity of photosynthetically usable light to the maximum photosynthesis achieved in the same experiment) allowed direct comparisons of photosynthetic responses of different species in different experiments over the same range of light intensities.

TABLE 1. Primary production estimates (gC $m^{-2}$ $day^{-1}$) at the edge of the continental shelf off Oregon by three methods. A = average $^{14}C$ measurement ± the half-range of two independent experiments; B = chlorophyll-light estimate (Chl-I) using one set of chlorophyll estimates each day and an assimilation ratio (AR) of 3.7; C = Chl-I calculated for 2-hr increments and integrated with sets of chlorophyll estimates throughout each day and AR determined every 2 hr each day.

| Date | Sky cover (*tenths*) | gC $m^{-2}$ $day^{-1}$ | | | % difference 100(B–A)/A | % difference 100(C–A)/A |
|---|---|---|---|---|---|---|
| | | A | B | C | D | E |
| Apr. 18, 62 | 0 | 0.68±0.09 | 1.00[b] | 0.65 | +47 | –4 |
| May 10, 62 | 2 | 1.05±0.17 | 1.73[b] | 1.15 | +65 | +10 |
| Mar. 24, 63 | 10 | 0.60±0.08 | 0.68 | 0.57 | +13 | –5 |
| May 24, 63 | 10 | 0.38±0.05 | 0.40 | 0.34 | +5 | –10 |
| Apr. 13, 65 | 10 | 0.27±0.05 | 0.33[b] | 0.24 | +22 | –11 |
| May 5, 67[a] | 10 | 0.74±0.08 | 0.76 | 0.69 | +3 | –7 |
| May 6, 67[a] | 9 | 0.68±0.11 | 0.73 | 0.76 | +7 | +12 |
| Aug. 19, 62 | 10 | 0.65±0.12 | 0.32[b] | 0.71 | –51 | +9 |
| July 3, 63 | 3 | 0.33±0.04 | 0.43[b] | 0.39[b] | +30 | +18 |
| Aug. 1, 63 | 7 | 0.46±0.08 | 0.46 | 0.40 | 0 | –13 |
| Oct. 2, 63 | 5 | 0.36±0.05 | 0.28[b] | 0.35 | –22 | –3 |
| June 3, 64 | 10 | 0.36±0.05 | 0.42[b] | 0.31 | +17 | –14 |
| July 1, 64 | 10 | 0.53±0.06 | 0.59 | 0.47 | +11 | –11 |
| Aug. 5, 64 | 0 | 0.75±0.14 | 1.30[b] | 0.85 | +73 | +13 |
| July 20, 65 | 9 | 0.59±0.10 | 0.55 | 0.57 | –7 | –3 |
| July 21, 65 | 5 | 0.64±0.16 | 0.68 | 0.70 | +6 | +9 |
| Sept. 6, 67[a] | 7 | 0.66±0.06 | 0.56[b] | 0.64 | +15 | –3 |
| Sept. 7, 67[a] | 6 | 0.57±0.08 | 0.59 | 0.65 | +4 | +14 |
| Nov. 16, 62 | 10 | 0.41±0.05 | 0.30[b] | 0.33[b] | –27 | –19 |
| Dec. 9, 62 | 6 | 0.18±0.04 | 0.35[b] | 0.26[b] | +94 | +44 |
| Jan. 14, 63 | 10 | 0.46±0.12 | 0.15[b] | 0.17[b] | –67 | –63 |
| Feb. 11, 63 | 0 | 0.78±0.09 | 0.97[b] | 0.76 | +24 | –3 |
| Nov. 28, 65 | 10 | 0.15±0.04 | 0.18 | 0.14 | +20 | –7 |
| Feb. 20, 66[a] | 2 | 0.42±0.06 | 0.49[b] | 0.39 | +17 | –7 |

[a]Chlorophyll-light estimates made before $^{14}C$ measurements.
[b]Outside the range of the $^{14}C$ experiments.

The I:T curves were corrected pyranometer records for selected days of the year. Each record of total measured radiation was halved to account for the fact that only 50% of the measured radiation is photosynthetically usable. The selected days represented the broadest possible range of I, and therefore covered all seasons. Because I presumably was common to both the P:I and I:T curves, P:T curves for each depth and each day were generated. Integration of the P:T curves over the complete daylight period yielded daily relative photosynthesis for each depth ($R_d$). When $R_d$ values for each of the selected days were plotted as functions of total daily surface radiation, smooth $R_d$ curves could be drawn. Once these curves were established, $R_d$ values for any day of the year could be estimated by knowing only the total daily surface radiation for the particular day.

When production estimates made with the basic equation from our own chlorophyll and light data were compared with concurrent in situ $^{14}C$ estimates, variable results were obtained (Table 1, columns A and B). Fourteen of the 24 chlorophyll-light estimates fell outside the ranges of the $^{14}C$ estimates. The average percentage difference between the chlorophyll-light estimates and the mean $^{14}C$ estimates was 27%, with the chlorophyll-light technique generally estimating higher (Table 1, column D). We felt that closer agreement between the two sets of estimates might be obtained by accounting for short-term variations in chlorophyll concentrations and production/chlorophyll ratios.

### Development of a New Equation

We developed a new equation in which primary production at each depth was estimated in 2-hr increments:

$$P_{d2} = R_{d2} \times C_{d2} \times A_2$$

where:

$P_{d2}$ = photosynthesis for a 2-hr increment of the daylight period at depth $d$ [as gC $m^{-3}$ $(2\ hr)^{-1}$].

$R_{d2}$ = relative photosynthesis for a 2-hr increment at depth $d$ [as R $m^{-3}$ $(2\ hr^{-1})$].

$C_{d2}$ = chlorophyll $a$ concentration for a 2-hr increment at depth $d$ [as (gChl $a$) $m^{-3}$].

$A_2$ = maximum photosynthesis/chlorophyll ratio for a 2-hr increment [as gC $hr^{-1}$ $(gChl\ a)^{-1}$]. At light saturation, $A_2$ is the assimilation number.

Values of $P_{d2}$ can be integrated over $d$ to yield $P_2$, with units of gC $m^{-2}$ $(2\ hr)^{-1}$. Finally, $P_2$ values are plotted against time of day and the resulting curve is integrated to yield daily production beneath a square meter of sea surface ($P$ = gC $m^{-2}$ $day^{-1}$). Two hours was the maximum resolution attempted in the above integration-by-parts technique, because $^{14}C$ measurements with which we were comparing our chlorophyll-light estimates represented 2-hr experiments.

The $R_{d2}$ curves were developed from the same three types of curves used to estimate $R_d$ in the basic equation; that is, P:I, I:T, and P:T curves. Presumably the P:I curve of Ryther (1956) applied to the phytoplankton in Oregon coastal waters. The P:T curves at each depth were integrated in 2-hr segments rather than for the full day, to yield $R_{d2}$ curves as in Fig. 1. Because the I:T curves were for clear days of different total radiation intensities, the resulting P:T curves were symmetrical about local noon. Because of this symmetry, any $R_{d2}$ value in the morning had a corresponding $R_{d2}$ value in the afternoon. Thus, $R_{d2}$ curves for 0400–0600 and 1800–2000 hr were identical, as were those for 0600–0800 and 1600–1800 hr, 0800–1000 and 1400–1600 hr, and 1000–1200 and 1200–1400 hr.

The $C_{d2}$ term corrected 1) for the rather systematic variation in chlorophyll concentration with time of day; 2) for unsystematic variation in chlorophyll concentration caused by clouds and fog which, for the time periods that they obscured the sun, diminished the incoming radiation from that expected from a clear, sunny sky; and 3) for spatial variation of chlorophyll in water moving past our fixed sampling station. Glooschenko et al. (1972) showed that the systematic diel variations at different depths were out of phase so that chlorophyll maxima and minima at different depths occurred at different times of the day. The $C_{d2}$ term accounted for these out-of-phase features of diel periodicity.

Use of a constant assimilation ratio was inadequate for production estimates in 2-hr increments during all seasons. During early morning and late afternoon, light saturation levels were never reached in the water. Curl and Small (1965) showed that gC $hr^{-1}$ $(gChl\ a)^{-1}$ at suboptimum light in Oregon waters varied considerably with time of day, depth in the water column, and possibly nutrient levels.

Maximum production/chlorophyll ratios in the water column at different times of the daylight day were plotted against time of day (Fig. 2). The relation could be described roughly by two curves,

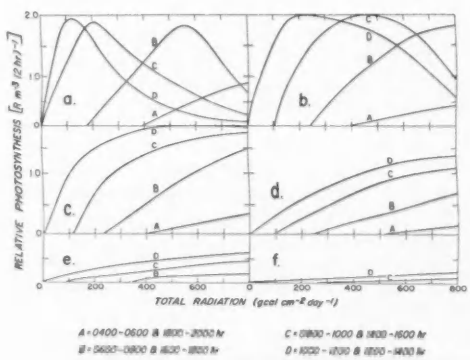


FIG. 1. Relative photosynthesis as a function of total daily radiation at six "light-depths" and for each 2-hr period in the daylight day ($R_{d2}$ curves). a = surface ($I_o$), b = 50% $I_o$, c = 25% $I_o$, d = 10% $I_o$, e = 4% $I_o$, f = 1% $I_o$.

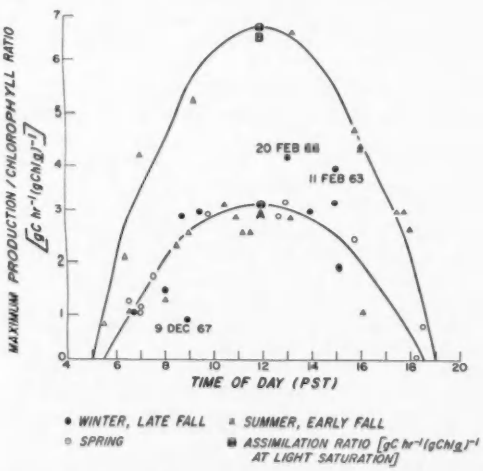


FIG. 2. Maximum production/chlorophyll ratios as a function of time of day under (A) non-upwelling conditions, and (B) upwelling conditions.

depending on the presence or absence of strong upwelling. In spring, late fall, winter, and summer with weak upwelling, the largest average production/chlorophyll ratio, assumed to be the ratio at light saturation (assimilation ratio), was approximately 3.1 at noon PST. In upwelled water, the curve was drawn through an assimilation ratio of 6.7. Any $A_2$ value could be read from the appropriate curve for the 2-hr period of interest.

There were some points that did not fit either curve in Fig. 2 well. The sampling dates of February 11, 1963 and February 20, 1966 were marked by unseasonably warm, sunny weather, and maximum production/chlorophyll ratios at the times of sampling were 3.9 and 4.2. The inflated ratios were of a magnitude that might be expected under conditions of weak summer upwelling, though the cause-effect relations were different; in February, increased ratios were the result of higher than average radiation coupled with preexisting nutrient sufficiency, whereas increased ratios in summer upwelling resulted from a nutrient "bonus" in the face of ample radiation. The ratio for December 9, 1962 at 0900 hr was much lower than other ratios at the corresponding time of day, and it is suggested that an error in determining the production/chlorophyll ratio might have occurred.

### Comparison of the Two Equations

Coastal production estimated by the new equation is shown in Table 1, column C. Three persons were responsible for calculations of production on different dates, so systematic individual error should have been minimized. The average percentage difference between production estimates by the new equation and mean $^{14}C$ estimates was 13%, an improvement by 52% over the 27% average difference between $^{14}C$ and basic equation estimates. The new equation was a better estimator of $^{14}C$ production than the basic equation on 17 of 24 dates. The basic equation estimated better on six dates, and estimates were equal on one date.

The poorest estimation by either equation occurred in winter. On December 9, 1962 an error possibly occurred in the determination of the production/chlorophyll ratio, as suggested earlier; however, the computed production was only 0.08 gC $m^{-2}$ $day^{-1}$ greater than the average $^{14}C$ estimate. On January 14, 1963 the $^{14}C$ estimate was considered to be in error (Small et al. 1972). On February 11, 1963 and February 20, 1966 the measured production/chlorophyll ratios were between the upwelling and non-upwelling curves (Fig. 2), and yet the new equation estimated within −3 and −7% of the $^{14}C$ values, respectively. The new equation estimated within +11 and +8% of $^{14}C$ estimates if the actual production/chlorophyll values for the two February dates were used rather than the $A_2$ values read from the curve.

The new equation was used to estimate offshore production on six dates (Table 2). Generally, estimation was better with the new equation than with the basic equation, although average percentage differences from $^{14}C$ estimation were larger offshore than near the coast, regardless of which equation was used. Average percentage difference between the basic equation estimates and the $^{14}C$ estimates was 31%, while the average percentage difference between the new equation estimates and $^{14}C$ estimates was 21%. Large percentage differences can result from small production differences in regions of low production, however.

TABLE 2. Primary production estimates (gC $m^{-2}$ $day^{-1}$) at offshore stations off Oregon by three methods. A, B, and C as in Table 1.

| Date | Distance from shore | Sky cover (*tenths*) | gC $m^{-2}$ $day^{-1}$ | | | % difference 100(B–A)/A | % difference 100(C–A)/A |
|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E |
| Apr. 12, 65 | 185 km | 2 | 0.44±0.07 | 0.52[b] | 0.41 | +18 | −7 |
| July 17, 65 | 530 km | 6 | 0.21±0.04 | 0.25 | 0.17 | +19 | −19 |
| July 18, 65 | 530 km | 0 | 0.37±0.08 | 0.51[b] | 0.33 | +38 | −11 |
| Sept. 28, 65 | 100 km | 10 | 0.21±0.07 | 0.27 | 0.29[b] | +29 | +38 |
| June 9, 66[a] | 29°12′N, 161°30′W | 0 | 0.10±0.02 | 0.15[b] | 0.13[b] | +50 | +30 |

[a]Chlorophyll-light estimates made before $^{14}C$ measurements.
[b]Outside the range of the $^{14}C$ experiments.

It is of some advantage to explore the conceptual changes brought about by the operational changes in the new equation, and to relate these conceptual changes to the basic equation. First, the $R_{d2}$ term of the new equation is conceptually the same as the $R_d$ term in the basic equation because both derive from the same P:I curves and from symmetrical I:T curves developed on clear days. All the $R_{d2}$ term does is to put relative photosynthesis on the same time basis (2 hr) as the chlorophyll estimates in the new equation. The $R_{d2}$ term does not compensate for production changes which might result from changes in the normal pattern of radiation expected from a clear sky during the day; i.e. there is no compensation for the "anomalous" condition of heavy morning overcast or fog followed by clear sky in the afternoon, for example.

The $C_{d2}$ term in the new equation is conceptually different from the $C_d$ term in the basic equation because it takes into account any changes in chlorophyll content of the water column during the day. Changes in chlorophyll content of the water column with time are considered to be the result of complex interactions involving diel periodicity, responses to gross light changes during days of "anomalous" light conditions, and any processes (advection, grazing, etc.) that might differentially remove phytoplankton cells from different depths in the water column. The $C_d$ term in the basic equation cannot account for such changes because it is evaluated at only one time during each day.

The new equation treats the assimilation number of the basic equation as a special case in a family of maximum production/chlorophyll ratios ($A_2$ values) throughout the day (Fig. 2). On a clear day the highest $A_2$ value (assimilation number) is reached at midday at the depth in the water column where light saturation of photosynthesis occurs. $A_2$ values in the early morning and late afternoon or early evening are lower than the assimilation number because 1) saturating light intensities are not achieved at the sea surface in early morning and evening, and 2) chlorophyll concentration is usually higher in early morning and evening than at midday. Moreover, on a clear day, morning and afternoon $A_2$ values fall away from the assimilation number in symmetrical fashion (Fig. 2).

The $A_2$ term in the new equation, like the $R_{d2}$ term, does not compensate for production changes which might result from "anomalous" light conditions during any given day. The $A_2$ term does put maximum production/chlorophyll estimates on the same 2-hr basis as the chlorophyll estimates, however. In addition, the $A_2$ values are reasonably sensitive to the nutrient environment, and can account for gross nutrient induced production increases like those found in upwelling areas. The precision with which the $A_2$ term can handle nutrient effects on production is not known, and herein lies a major source of error in the new equation: the selection of $A_2$ values from the proper curve in Fig. 2. Correct selection demands some a priori knowledge of the processes affecting the area being sampled. In all probability a family of curves similar to the two curves in Fig. 2 would lend more precision to determination of $A_2$ over a range of nutrient conditions, but we have no data from which to construct any curves other than those shown in Fig. 2. The data points for February 11, 1963 and February 20, 1966 possibly are applicable to a curve intermediate between the two shown, as indicated earlier.

A major disadvantage of the new chlorophyll-light technique is that the computations take more time than those involved in the basic equation. Also, because there is more dependence on curve reading and more numerical integration in the new technique, the computational error is probably greater than with the basic equation; nevertheless, the improvement in estimation of the equation components on a 2-hr basis generally seems to more than offset any increase in computational error.

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CURL, H., JR., AND L. F. SMALL. 1965. Variations in photosynthetic assimilation ratios in natural, marine phytoplankton communities. Limnol. Oceanogr. 10, Suppl.: R67–R73.

GLOOSCHENKO, W. A., AND H. CURL, JR. 1971. Influence of nutrient enrichment on photosynthesis and assimilation ratios in natural North Pacific phytoplankton communities. J. Fish. Res. Bd. Canada 28: 790–793.

GLOOSCHENKO, W. A., H. CURL, JR., AND L. F. SMALL. 1972. Diel periodicity of chlorophyll *a* concentration in Oregon coastal waters. J. Fish. Res. Bd. Canada 29: 1253–1259.

RYTHER, J. H. 1956. Photosynthesis in the ocean as a function of light intensity. Limnol. Oceanogr. 1: 61–70.

1969. Photosynthesis and fish production in the sea. Science 166: 72–76.

RYTHER, J. H., AND C. S. YENTSCH. 1957. The estimation of phytoplankton production in the ocean from chlorophyll and light data. Limnol. Oceanogr. 2: 281–286.

SMALL, L. F. 1967. On the standardization of $C^{14}$ for primary production in aquatic environments. Iowa State J. Sci. 42: 63–71.

SMALL, L. F., H. CURL, JR., AND W. A. GLOOSCHENKO. 1972. Effects of solar radiation and upwelling on daily primary production off Oregon. J. Fish. Res. Bd. Canada 29: 1269–1275.

STEEMAN-NIELSEN, E. 1952. The use of radio-active carbon for measuring organic production in the sea. J. Cons. Cons. Perma. Int. Explor. Mer 18: 117–140.

TALLING, J. F. 1957. The phytoplankton population as a compound photosynthetic system. New Phytol. 56: 133–149.

THOMAS, W. H. 1970a. On nitrogen deficiency in tropical Pacific oceanic phytoplankton: Photosynthetic parameters in poor and rich water. Limnol. Oceanogr. 15: 380–385.

1970b. Effect of nitrate and ammonia concentration on chlorophyll increases in natural tropical Pacific phytoplankton populations. Limnol. Oceanogr. 15: 386–394.

VOLLENWEIDER, R. A. 1965. Calculation models of photosynthesis-depth curves and some implications regarding day rate estimates in primary production measurements, p. 427–457. *In* C. R. Goldman [ed.] Primary productivity in aquatic environments. Mem. Ist. Ital. Idrobiol. Suppl. 18, University of California Press, Berkeley, Calif.

# Effects of Solar Radiation and Upwelling on Daily Primary Production off Oregon

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SMALL, L. F., H. CURL, JR., AND W. A. GLOOSCHENKO. 1972. Effects of solar radiation and upwelling on daily primary production off Oregon. J. Fish. Res. Bd. Canada 29: 1269–1275.

Primary production was measured in units of gC $m^{-2}$ $day^{-1}$ (=P) and gC $(gChla)^{-1}$ $day^{-1}$ on a per meter squared basis ($=P_{Chl}$) for 4 years in Oregon coastal waters and offshore areas. P, and particularly $P_{Chl}$, were functions of daily photosynthetically usable radiation inshore except in summer, and offshore apparently during all seasons. Values of P and $P_{Chl}$ for a given daily input of usable radiation were higher inshore than offshore, probably because of more efficient use of penetrating light by the relatively heavy coastal phytoplankton populations in nutrient-rich water. Evidence is presented that inshore phytoplankton populations in winter are acclimated to low light relative to inshore populations in spring.

P and $P_{Chl}$ are related to the interaction of daily radiation and degree of upwelling in Oregon coastal waters in summer. P is a function of photic depth offshore and inshore, except in summer. Probably the presence of large quantities of non-photosynthesizing detritus, even in areas of upwelling, destroys the relation in summer. There apparently is no simple relation involving daily production and certain significant environmental factors which will allow prediction of daily production in all regions of the ocean during all seasons.

SMALL, L. F., H. CURL, JR., AND W. A. GLOOSCHENKO. 1972. Effects of solar radiation and upwelling on daily primary production off Oregon. J. Fish. Res. Bd. Canada 29: 1269–1275.

Les auteurs ont mesuré, durant quatre années, la production primaire des eaux côtières et du large de l'Orégon. Ils ont exprimé ces mesures en unités de gC $m^{-2}$ $jour^{-1}$ (=P) et de gC $(gChla)^{-1}$ $jour^{-1}$ sur une base de mètre au carré ($=P_{Chl}$). P, et surtout $P_{Chl}$, sont fonctions de la radiation journalière utilisable pour la photosynthèse, vraisemblablement en toute saison dans les eaux du large, mais non en été dans les eaux côtières. Les valeurs de P et de $P_{Chl}$, pour un apport journalier de radiation utilisable donné, sont plus élevées près de la côte qu'au large, sans doute à cause de l'utilisation plus efficace de la lumière par les populations côtières de phytoplancton relativement denses, dans des eaux riches en substances nutritives. Les auteurs démontrent qu'en hiver, les populations côtières de phytoplancton sont acclimatées à une faible radiation, comparativement aux populations de printemps.

P et $P_{Chl}$ sont liées à l'interaction de la radiation journalière avec le degré de remontée d'eaux profondes près de la côte de l'Orégon en été. P est fonction de la profondeur à laquelle les rayons solaires pénètrent au large et près de la côte, sauf en été. Les détritus non photosynthétiques, présents en grandes quantités, même dans les secteurs de remontée d'eaux profondes, détruisent probablement cette relation en été. Il ne semble pas y avoir de relation simple entre la production journalière et certains facteurs importants du milieu, relation qui permettrait de prédire cette production dans toutes les régions de l'océan en toute saison.

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THE typical mid-latitude seasonal cycle of primary productivity in the sea usually presented consists of a large production peak in spring, low production in summer, a small peak in fall, and low production again in winter (Raymont 1963). Low production in summer generally is the result of a stabilized water column which slows resupply of nutrients to surface waters, and the winter low mainly is the result of decreased solar radiation and increased depth of mixing. In coastal regions in which upwelling occurs in summer, however, nutrient concentration can remain high, and primary productivity should be roughly a function of degree of upwelling if solar radiation remains relatively constant. If

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solar radiation varies considerably, because of changing weather conditions, production should reflect these changes as well as changes in the rate of upwelling.

Primary production estimates were made in the sea off Oregon for 4 consecutive years in an effort to establish the major factors responsible for the fertility of the water in all seasons. There are no previous published measurements of primary production off Oregon except those reported by Anderson (1964), who measured simulated in situ productivity at random positions off the coast during 1961 and part of 1962.

## Methods

Productivity was measured as often as feasible from 1962 through early 1966 at a station 46 km west of Newport, Oreg. The station, at 44°39′N, 124°38′W, was approximately at the edge of the continental shelf and was considered representative of the coastal area. In addition to the single station, several other stations were occasionally occupied within 5 km of the 46 km station, and many productivity estimates were made at one time of day during different days by simulated in situ experiments on the deck of the ship. The latter experiments served as a rough check on the spatial variability of production in the region of interest. The simulated in situ data were not used in the analysis of daily production, however, because they were, in all cases, single estimates at different times of different days. However, if the single estimates did not agree within ±20% of the corresponding estimate at the 46 km station, the data at the 46 km station were not used to estimate daily production.

The movement of surface waters along the Oregon coast (Burt and Wyatt 1964) is generally in a southerly direction from June through August, and northerly from about mid-October through mid-March. Late March through May, and September through early October, are usually transition periods during which the surface currents vary in direction. In summer, surface water is moved offshore due to prevailing northwest winds (Collins 1964) and is replaced along the coast by colder, more saline water upwelled from greater depths. From mid-October through mid-March the northward flowing Davidson Current is found along the coast, due largely to the prevailing southerly winds. Surface temperatures in the Davidson Current can be higher than those in summer water during times of upwelling. Maughan (1963) and Collins (1964) both reported onshore components for the surface water during these winter months.

The most outstanding hydrodynamic feature of Oregon coastal waters in summer is upwelling. Though degree of upwelling is a difficult feature to measure, we used two independent methods which agree very well with each other for our data. In the first method, the slopes of the 25.5 and 26.0 sigma-$t$ ($\sigma_t$) contour lines in the upper 100 meters off Newport were evaluated. Collins (1964) has indicated that the 25.5 and 26.0 $\sigma_t$ contours delineate the permanent pycnocline off Oregon. In winter, with no upwelling, the selected contours were always horizontal or tilted slightly downward in a shoreward direction (Fig. 1a). However, in summer the contours were inclined upward in varying degrees towards shore. The average angle of deflection of the lines from the horizontal, as they passed through the 46 km station (Fig. 1b), was used as a measure of upwelling. The maximum average angle ($U_{max}$) was found in July 1962. All other average angles (U) were expressed relative to the maximum angle to yield $U/U_{max}$. With this technique, degree of upwelling was compared roughly among different years of the study, and among months of the same year (Fig. 2). Finer resolution than monthly comparison was not warranted because of the time intervals between estimates of $\sigma_t$. Also, because of lack of extreme precision in contouring $\sigma_t$, and thus in establishing the angles,

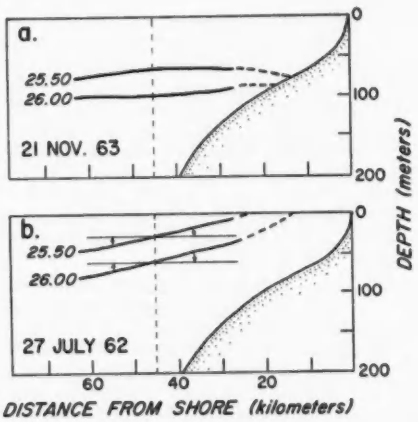


FIG. 1. Representative contour lines of $\sigma_t = 25.5$ and 26.0 in the upper 150 m off Newport, Oregon, in winter (a) and in summer (b). The average angle of departure from horizontal at the 46 km station was used as one measure of upwelling.

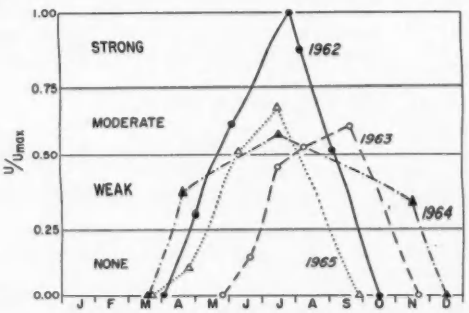


FIG. 2. Relative upwelling ($U/U_{max}$) off Newport, Oregon for the 4 years of the study, calculated by Method 1 (see text).

only four categories of upwelling were recognized: no upwelling ($U/U_{max} = 0.00$–$0.25$), weak upwelling (0.26–0.50), moderate upwelling (0.51–0.75), and strong upwelling (0.76–1.00).

The second method for assessing degree of upwelling involved comparisons of Ekman offshore transport in the upper 100 m in the region of the 46 km station (specifically, at 45°N, 125°W) during the summer months. Transports in units of metric tons $sec^{-1}$ $km^{-1}$ were developed from geostrophic wind calculations using pressure field changes rather than actual wind measurements. Data were available every month of every year from the Environmental and Fishery Forecasting Center, National Marine Fisheries Service, Fleet Numerical Weather Control, Monterey, Calif. In such calculations we assumed that water transported offshore in the upper 100 m was replaced by water upwelled from below the permanent pycnocline (below 100 m), and therefore changes in Ekman transport were direct measures of degree of upwelling. The maximum transport ($U_{max}$) was found in July, 1962 (which was also the date on which the maximum average deflection of the permanent pycnocline occurred at the 46 km station). All other Ekman transports were expressed relative to the maximum transport to yield $U/U_{max}$, and the $U/U_{max}$ ratios were then plotted by month and year (not illustrated). Regression of $U/U_{max}$ values by method 2 (Ekman transport) against $U/U_{max}$ values by method 1 (pycnocline tilt) on corresponding dates yielded a slope of 0.96, which was very close to the theoretical slope of 1.00 (Fig. 3). However, there was appreciable variation about the regression line, as exemplified by a correlation coefficient of 0.49.

It is significant to mention that we used estimates of degree of upwelling based on measurements of conservative properties of the water. Though it is the nutrient content of the upwelled water that directly affects phytoplankton production, the fact that nutrient content is extremely nonconservative in upper waters negates the use of nutrient contours for calculating degree of upwelling. We assumed that, in the absence of biological utilization, the transport of dissolved nutrients into upper waters and subsequently offshore would follow the transport of water as deduced from conservative properties. We knew also that the nutrient reservoir from which the upwelled nutrients originated did not change over the time course of the upwelling season, so that any nonbiological changes in nutrient amounts delivered to surface waters were functions of changes in the rate of replacement of surface waters moved offshore and not functions of changing reservoir size. Concentrations of nitrate-nitrogen remained at 20$\mu$M $liter^{-1}$ at the bottom edge of the permanent pycnocline offshore.

The methods for estimating chlorophyll *a*, incident and penetrating radiation, and primary production were given in other papers (Glooschenko et al. 1972; Small et al. 1972). In addition to reporting $^{14}C$ production estimates in terms of gC $m^{-2}$ $day^{-1}$ (P) we determined chlorophyll-specific production ($P_{Chl}$) with units of gC $(gChl\,a)^{-1}$ $day^{-1}$ computed on a per meter squared basis. The $P_{Chl}$ estimates were somewhat similar to the efficiency indices used by Mandelli and Burkholder (1966), and afforded us another means of evaluating light and upwelling effects on primary production.

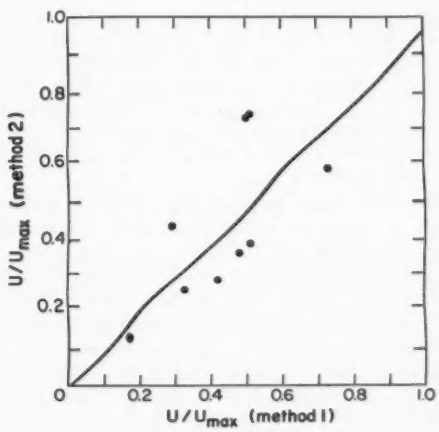


FIG. 3. Relation between $U/U_{max}$ calculated by methods 1(X) and 2(Y) in summer off Oregon [$Y = 0.96X - 0.01$, $r = 0.49$].

## Results

Total daily primary production (P) varied seasonally between about 0.1 and 1.0 gC $m^{-2}$ $day^{-1}$ and chlorophyll-specific production ($P_{Chl}$) between 0.2 and 1.3 gC gChl $a)^{-1}$ $day^{-1}$ (Fig. 4). In most instances, photosynthetically usable surface radiation on the days of sampling approached the weekly averages of usable radiation, the most severe deviations occurring in spring and summer.

Total daily production in spring and winter, and particularly daily chlorophyll-specific production, were functions of daily usable radiation (Figs. 5a, c),

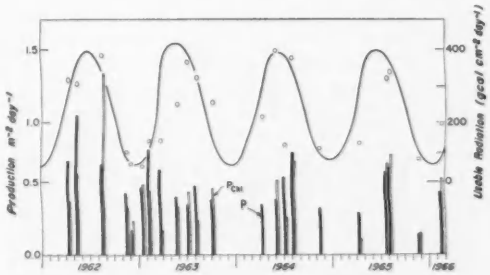


FIG. 4. Total daily production (P = gC $m^{-2}$ $day^{-1}$), daily chlorophyll-specific production ($P_{Chl}$ = gC $(gChl\,a)^{-1}$ $day^{-1}$), and total daily usable radiation incident to the sea surface (○) on the sampling dates. Mean weekly usable radiation is shown as a continuous curve.

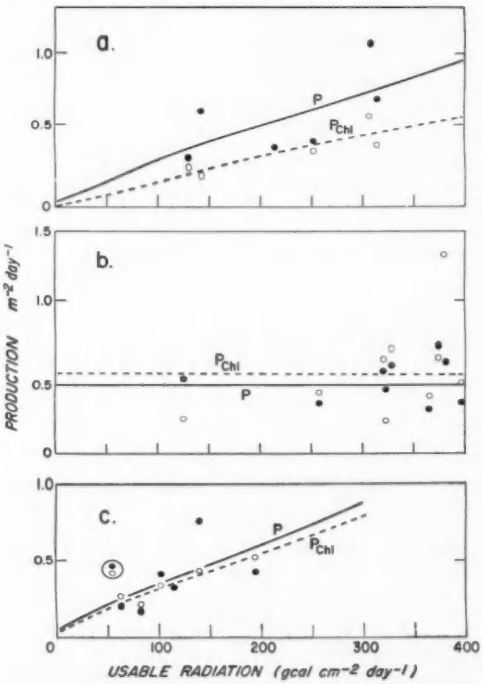


FIG. 5. Relations among P, $P_{Chl}$, and total daily usable radiation at the sea surface (R) at the 46 km station in (a) spring ($P = 0.03 + 0.0023R$, $r = 0.63$, $P_{Chl} = 0.00 + 0.0019R$, $r = 0.81$); (b) summer (mean $P = 0.49 \pm 0.13$ SD, mean $P_{Chl} = 0.57 \pm 0.35$ SD); (c) winter without circled Jan. 14, 1963 data points ($P = 0.04 + 0.0029R$, $r = 0.58$, $P_{Chl} = 0.03 + 0.0026R$, $r = 0.95$).

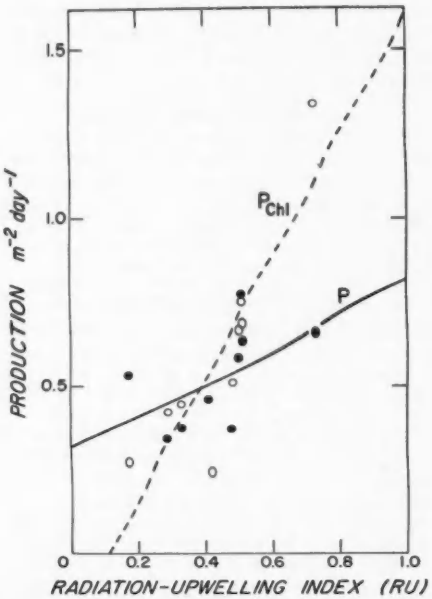


FIG. 6. Relationships among P, $P_{Chl}$, and a radiation-upwelling index (RU) calculated by Method 1 (see text) at the 46 km station in summer ($P = 0.30 + 0.50RU$, $r = 0.54$, $P_{Chl} = 1.80RU - 0.22$, $r = 0.87$).

but no relation was observed in summer between production and usable radiation (Fig. 5b). Values of P and $P_{Chl}$ in summer were $0.49 \pm 0.13$ gC $m^{-2}$ $day^{-1}$ and $0.57 \pm 0.35$ gC (gChl *a*)$^{-1}$ $day^{-1}$, respectively, where $\pm 0.13$ and $\pm 0.35$ set the limits of one standard deviation. Production in summer also was not correlated with relative upwelling ($U/U_{max}$) alone determined by either method. However, the product of relative upwelling and relative usable radiation yielded a radiation-upwelling index with which P, and particularly $P_{Chl}$, were correlated. The correlations were highest (Fig. 6) with the radiation-upwelling index developed from $U/U_{max}$ computed from upwelling method 1 (pycnocline tilt), but method 2 (Ekman transport) also gave acceptable results (not illustrated). The regression equations and correlation coefficients ($r$) for method 2 were: $P = 0.34 + 0.51$ RU, with $r = 0.52$; and $P_{Chl} = 1.92$ RU $- 0.33$, with $r = 0.72$. The term for relative usable radiation on a particular day was the ratio of usable radiation on that day to maximum usable radiation measured during the period of study (maximum = 396.4 gcal $cm^{-2}$ $day^{-1}$ on June 3, 1964).

Primary production out of the region of upwelling in summer should be mainly a function of daily usable radiation, as in spring and winter. Four sets of in situ $^{14}C$ experiments performed at stations 100–530 km from shore, and one performed in the middle of the North Pacific (29°12′N, 161°30′W), were analyzed. Both P and $P_{Chl}$ were correlated significantly with daily usable radiation, over a range of radiation values from about 200 to 310 gcal $cm^{-2}$ $day^{-1}$ (Fig. 7).

A log-log curve fit to the offshore data and the winter and spring inshore data on total daily production as a function of photic depth (Fig. 8) had a correlation coefficient of 0.75. The relation was similar to that in the tropical and subtropical Atlantic (Steeman-Nielsen 1958), although the ranges of production values and of photic depths were somewhat different. Total inshore production off Oregon in summer was not a function of photic depth, the measured production being less than that indicated by the winter-spring and offshore curve.

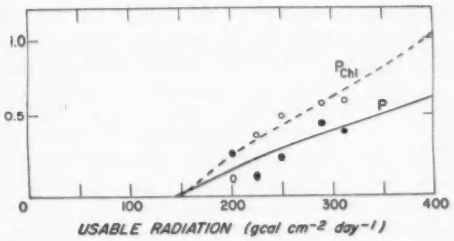


FIG. 7. Relations among P, $P_{Chl}$, and R at offshore stations ($P = 0.0025R - 0.35$, $r = 0.78$, $P_{Chl} = 0.0041R - 0.61$, $r = 0.93$).

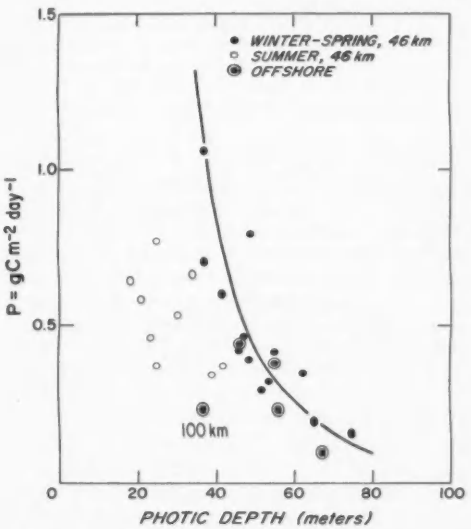


FIG. 8. Relations between P and photic depth inshore and offshore during all seasons. The line was fitted through all data except those in summer at the 46 km station.

## Discussion

The Oregon coastal region ranks with other productive areas in the world ocean. Strickland (1960) tabulated the results of $^{14}C$ based productivity measurements made by numerous investigators in different regions. Generally, values ranged from about 0.1 to 1.5 gC $m^{-2}$ (24 hr)$^{-1}$ in coastal waters (depending to some extent upon latitude and season), and from 0.01 to 0.5 gC $m^{-2}$ (24hr)$^{-1}$ in offshore waters. These values can be compared with our values of P, which ranged between 0.1 and 1.0 gC $m^{-2}$ $day^{-1}$ (Fig. 4). Values given on a 24-hr basis might be somewhat less than those on a daylight day basis because the former presumably are corrected for nightly respiratory utilization of carbon synthesized during the day.

Daily chlorophyll-specific production in Oregon waters ranged from 0.2 to 1.3 gC (gChl *a*)$^{-1}$ $day^{-1}$ on a per square meter basis (Fig. 4). To our knowledge the only other estimates of $P_{Chl}$ were made in the Gerlache and Bransfield Straits of Antarctica (Mandelli and Burkholder 1966), where values ranged from 4.7 to 18.1, and in the central Subarctic Pacific region just south of the Aleutians (Larrance 1971), where values ranged from about 5.0 to 30.0. Total daily production was generally similar in the Antarctic, in the Subarctic Pacific, and in Oregon coastal waters, but obviously the Antarctic and subarctic Pacific populations were much more efficient in attaining it.

The regression line for $P_{Chl}$ in winter was rotated counter-clockwise relative to the regression line for $P_{Chl}$ in spring (Figs. 5a, c), indicating higher daily chlorophyll-specific production in winter at comparable light intensities. Such a relationship suggested that the winter phytoplankton community was adapted to low light intensity. If not expressed on a per chlorophyll basis, winter and spring production rates were relatively similar at given light levels. The data points for January 14, 1963 (Fig. 5c) were considered to be the result of a questionable series of $^{14}C$ measurements on that date.

The slope of the regression line for P offshore (Fig. 7) was not significantly different from the slopes of the comparable regression lines inshore in winter and spring (Figs. 5a, c). However, the regression line for the offshore data intersected the abscissa at about 150 gcal $cm^{-2}$ $day^{-1}$, while the lines for inshore data cut approximately through the origin. The result was that total production offshore was less than that inshore at comparable light levels. We interpreted this difference to be largely the result of more efficient use of penetrating light by heavy coastal populations in nutrient-rich water, relative to meager offshore populations in nutrient-impoverished water. Chlorophyll-specific production also was higher inshore than offshore at comparable light levels, again presumably the result of more effective utilization of penetrating radiation by the denser inshore populations. However, $P_{Chl}$ offshore generally was greater than P offshore, which was the opposite of the relation observed inshore; thus, even though more efficient use of the total light penetrating the water was made by the total population inshore, more efficient use of the fraction of total penetrating light that impinged on each cell was made by the population offshore.

The fact that values of P and $P_{Chl}$ were functions of total daily usable radiation except inshore in

summer indicated that measured surface radiation values could be used to predict production in non-upwelling situations. Total daily production is the most useful measurement, but in Oregon water P had the lowest correlation with daily usable radiation. Daily chlorophyll-specific production was more precisely correlated with daily radiation, but several measurements of chlorophyll *a* concentration in the photic zone over the daylight period would be required before $P_{Chl}$ could be estimated. So many chlorophyll measurements would minimize the usefulness of the relation between $P_{Chl}$ and light; one might as well determine in situ $^{14}C$ productivity directly as measure chlorophyll fluctuations and daily radiation and then use the relation between the two to estimate production. Generation of chlorophyll values as suggested by Glooschenko et al. (1972), after measurement of one series with depth, would be one means of making the production-light relation useful.

Total daily production in winter and spring in Oregon coastal waters, and in offshore areas, can be predicted roughly from photic depth measurements. As with prediction from daily usable radiation, prediction from photic depth measurements would be poor in coastal waters in summer (Fig. 8).

We suspected that non-photosynthesizing detritus was particularly heavy in inshore waters, even in areas of upwelling, and that such detritus contributed greatly to the decreased photic depth. Production would be a function of photic depth only insofar as photosynthesizing phytoplankton largely controlled the attenuation of light in the water; in this sense, it would be more appropriate to consider photic depth a function of production, rather than the reverse. Small and Curl (1968) showed that the extinction of light in Oregon coastal waters was a function of chlorophyll *a* concentration in all seasons but summer, at which time non-chlorophyllous detritus greatly affected light attenuation. Detrital material also might have been responsible for the relatively shallow photic depths and low production at the 100 km station (Fig. 8). The 100 km station was the farthest inshore of the offshore stations, and was over the continental slope off Newport. Conceivably summer production at this station was allied more to coastal than to offshore production.

Although the relation between summer primary production (particularly chlorophyll-specific production) and the radiation-upwelling index appeared to be satisfactory for the data presented (Fig. 6), there is little reason at this time to expect the relation to hold universally. Simple expressions of relative upwelling such as $U/U_{max}$, calculated from monthly data on pycnocline slope or Ekman offshore transport, ignore some possibly significant features of an upwelling-production relation; for example, they ignore possible effects of longshore current velocities, and they average out short-term effects such as daily or weekly shifts in the wind field. They also ignore time lags between onset of upwelling and development of phytoplankton populations, and time lags between sites of maximum vertical velocity and sites of maximum phytoplankton production. Recent data of Mooers and Smith (1967), Collins et al. (1968), Mooers et al. (1968), and Small and Ramberg 1972, for example, indicate that upwelling in surface waters off Oregon is expressed most strongly within the first 10 or 15 km of the coast, not at 46 km. Nevertheless, even with all of the potential difficulties in using monthly upwelling estimates in conjunction with daily production and radiation estimates, the relationship between production and the radiation-upwelling index was good for Oregon waters.

There apparently is no simple relationship involving daily production and certain significant environmental factors which will allow prediction of daily production in all regions of the ocean during all seasons. The equations developed by Ryther (1956), Ryther and Yentsch (1957), and Vollenweider (1965) probably are usable in most waters bordering on nutrient sufficiency, but might not be usable in areas of upwelling and divergences, and on days with a highly variable radiation flux and chlorophyll concentration during the day. If upwelling areas are as significant in terms of primary production and fish production as indicated by Ryther (1969), then precise measurements of primary production in those areas are critical. In another paper (Small et al. 1972), we attempt to modify existing equations to account for the effects of environmental fluctuations on primary production in upwelling areas.

## Acknowledgments

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ANDERSON, G. C. 1964. The seasonal and geographic distribution of primary productivity off the Washington and Oregon coasts. Limnol. Oceanogr. 9: 284–302.

BURT, W. V., AND B. WYATT. 1964. Drift bottle observations of the Davidson Current off Oregon, p. 156–165. *In* Studies on oceanography. Univ. Tokyo Press, Tokyo, Japan.

COLLINS, C. A. MS 1964. Structure and kinematics of the permanent oceanic front off the Oregon coast. M.Sc. Thesis. Oregon State Univ., Corvallis, Oreg. 73 p.

COLLINS, C. A., C. N. K. MOOERS, M. R. STEVENSON, R. L. SMITH, AND J. G. PATTULLO. 1968. Direct current measurements in the frontal zone in a coastal upwelling region. J. Oceanogr. Soc. Jap. 24: 295–306.

GLOOSCHENKO, W. A., H. CURL, JR., AND L. F. SMALL. 1972. Diel periodicity of chlorophyll a concentration in Oregon coastal waters. J. Fish. Res. Bd. Canada 29: 1253–1259.

LARRANCE, J. D. 1971. Primary production in the mid-Subarctic Pacific Region, 1966–68. Fish. Bull. 69: 595–613.

MANDELLI, E. F., AND P. R. BURKHOLDER. 1966. Primary productivity in the Gerlache and Bransfield Straits of Antarctica. J. Mar. Res. 24: 15–27.

MAUGHAN, P. M. 1963. Observations and analysis of ocean currents above 250 meters off the Oregon coast. M.Sc. Thesis. Oregon State Univ., Corvallis, Oreg. 49 p.

MOOERS, C. N. K., L. M. BOGERT, R. L. SMITH, AND J. G. PATTULLO. 1968. A compilation of observations from moored current meters and thermographs (and of complementary oceanographic and atmospheric data). Dept. Oceanogr., Oregon State Univ., Corvallis, Oreg. Data Rep. 30 (Ref. 68–5, Mimeo.). 98 p.

MOOERS, C. N. K., AND R. L. SMITH. 1967. Dynamic Structure in an upwelling frontal zone (abstract). Trans. Amer. Geophys. Union 48: 125–126.

RAYMONT, J. E. G. 1963. Plankton and productivity in the oceans. Macmillan and Company, Limited, New York, N.Y. 660 p.

RYTHER, J. H. 1956. Photosynthesis in the ocean as a function of light intensity. Limnol. Oceanogr. 1: 61–70.

1969. Photosynthesis and fish production in the sea. Science 166: 72–76.

RYTHER, J. H., AND C. S. YENTSCH. 1957. The estimation of phytoplankton production in the ocean from chlorophyll and light data. Limnol. Oceanogr. 2: 281–286.

SMALL, L. F., AND H. CURL, JR. 1968. The relative contribution of particulate chlorophyll and river tripton to the extinction of light off the coast of Oregon. Limnol. Oceanogr. 13: 84–91.

SMALL, L. F., H. CURL, JR., AND W. A. GLOOSCHENKO. 1972. Estimates of primary production off Oregon using an improved chlorophyll-light technique. J. Fish. Res. Bd. Canada 29: 1261–1267.

SMALL, L. F., AND D. A. RAMBERG. 1972. Chlorophyll *a*, carbon and nitrogen in particles from a unique coastal environment, p. 00–00. *In* J. D. Costow, Jr. [ed.]. Proceedings international symposium on the fertility of the sea. Sao Paulo, Brazil. (In press)

STEEMAN-NIELSEN, E. 1958. Light and organic production in the sea. Rapp. Proces-Verbaux Reunions Cons. Perma. Int. Explor. Mer 144: 141–148.

STRICKLAND, J. D. H. 1960. Measuring the production of marine phytoplankton. Fish. Res. Board Can. Bull. 122: 172 p.

VOLLENWEIDER, R. A. 1965. Calculation models for photosynthesis–depth curves and some implications regarding day rate estimates in primary production measurements, p. 425–457. *In* C. R. Goldman [ed] Primary productivity in aquatic environments. Mem. Inst. Ital. Idrobiol., 18 suppl. Univ. California Press, Berkeley, Calif.

# Timing of Changes in the Blood, Morphology, and Behavior of *Petromyzon marinus* During Metamorphosis

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BEAMISH, F. W. H., AND I. C. POTTER. 1972. Timing of changes in the blood, morphology, and behavior of *Petromyzon marinus* during metamorphosis. J. Fish. Res. Bd. Canada 29: 1277–1282.

Morphological, biochemical, and behavioral criteria, from samples collected from 1969 to 1971, showed that metamorphosis in *P. marinus* is of a relatively short duration. In an analysis of sequential catches during 1971, the first external signs of transformation were observed only in samples caught between July 12 and 26, illustrating the marked synchrony characteristic of this and subsequent developmental stages. Relative increases in size of the disc and snout were most marked from mid-August to late October. Polyacrylamide gel electrophoresis showed that the change-over from larval to adult hemoglobins took place mainly between early August and late October, the beginning and peak of this process being characterised by reduced hematocrit values. Over 95% of the animals captured at various times in the field commenced feeding on suckers (*Catostomus commersoni*) in the laboratory by late November or early December.

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Les critères morphologiques, biochimiques et de comportement d'échantillons recueillis en 1969, à 1971 démontrent que la métamorphose de *P. marinus* est de durée relativement courte. Dans des prises effectuées successivement en 1971, les premiers signes extérieurs de métamorphose ne sont apparus qu'entre le 12 et le 26 juillet, ce qui démontre le synchronisme caractéristique de ce stade de développement et du suivant. La croissance relative du disque buccal et du museau est plus prononcée entre la mi-août et la fin d'octobre. L'électrophorèse sur gel polyacrylamide démontre que le changement des hémoglobines de la larve à celles de l'adulte a lieu surtout du début d'août à la fin d'octobre, le début et la pointe de ce processus étant caractérisés par des valeurs d'hématocrite réduites. Plus de 95% des sujets capturés à différentes périodes sur le terrain ont commencé à se nourrir de catostomes noirs communs (*Catostomus commersoni*) au laboratoire vers la fin de novembre ou le début de décembre.

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THERE have been only a few attempts to analyse the timing of the various metamorphic changes in lampreys. Such documentation is essential for an understanding of the factors involved in the onset of feeding in parasitic lampreys. Balabai (1948) and Manion and Stauffer (1970) have provided information on morphological changes in the brook lamprey, *Eudontomyzon mariae*, and the landlocked parasitic species *Petromyzon marinus*, respectively, while electrophoretic studies have established that changes in at least some of the plasma proteins and hemoglobin components occur during the metamorphosis of parasitic and nonparasitic lampreys (Adinolfi and Chieffi 1958; Adinolfi et al. 1959; Rall et al. 1961; Manwell 1963; Uthe and Tsuyuki 1967; Potter and Nicol 1968).

A problem encountered in any discussion of metamorphosis in lampreys is that different criteria have been employed to define its completion. For example, in *P. marinus*, Gage (1928), Applegate and Brynildson (1952), and Wigley (1959) have used emergence from the substrate, commencement of downstream migration, or onset of parasitic feeding whereas Vladykov (1949) used attainment of particular quantifiable morphological characters.

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In a more recent study, Manion and Stauffer (1970) used a combination of morphological and behavioral characters. In this study on *P. marinus* from a tributary of Lake Ontario, changes in electrophoretic patterns of blood hemoglobins, morphology, and behavior are used to elucidate the timing of the various phases of metamorphosis.

## Materials and Methods

Ammocoetes, metamorphosing, and spawning-run stages of *P. marinus* were caught by electric fish-shocker in Shelter Valley Creek between February 1969 and October 1971; during 1971, weekly samples were collected. The watershed has an approximate length of 17 km. Adults were reared in the laboratory from the macrophthalmia stage and fed on a diet of white suckers, *Catostomus commersoni*, and carp, *Cyprinus carpio*.

The transforming and macrophthalmia stages were initially separated by sex. However, since significant differences were not found in either weight or length between males and females, the data were pooled with respect to sex. Thus, the means of length and weight ($\pm$ 1 SE) for metamorphosing males and females measured live were, respectively: 140.9 $\pm$ 1.87, 142.7 $\pm$ 1.33 mm and 4.28 $\pm$ 0.174, 4.41 $\pm$ 0.147 g.

Within 3 hours of capture and prior to sampling for blood analysis, lampreys were anaesthetised with carbon dioxide. Blood was withdrawn from the hearts of ammocoetes and metamorphosing stages in heparinized capillaries and from adults by inserting a heparinized syringe into the anterior dorsal aorta. Capillaries were closed with a commercial sealant and spun at 12,000 rpm for 5 min and hematocrits measured on a microcapillary reader.

Blood cells for electrophoresis were centrifuged, washed three times with 0.9% NaCl, and hemolysed by the addition of a small amount of double distilled water, the cell debris being removed by centrifugation. The supernatant hemoglobin solution was matched for color against a 10% bovine hemoglobin color standard by adding further water. Samples of various concentrations, equivalent to 10–50 $\mu$g of bovine hemoglobin, were placed on 7% polyacrylamede gels (polymerized by light) in tubes of 8 cm long and 0.5 cm internal diameter. A Model 490 Isco power supply provided for 45 min at 4 C a constant current of 4.3 mA per tube. Electrophoresis was carried out separately with three buffers: (a) 48.0 g Tris and 28.8 g of glycine in 3 liters of water, *p*H 9.2 (S. E. Dixon personal communication), (b) 18.5 g boric acid and 2.4 g NaOH in 1 liter of water, *p*H 8.5 (Smithies 1959) and (c) 16.0 g Tris, 1.6 g $Na_2$ EDTA and 0.61 g boric acid in 2 liters of water, *p*H 9.2 (Li and Riggs 1970). Gels were stained with either amido black or O-dianisidine (Owen and Smith 1961). Electrophoresis of hemoglobins was carried out during 1971 on samples from 10 ammocoetes each week, from 10 metamorphosing animals each week from July to late October, and from approximately 50 adults collected in the various months of the year.

The various stages of metamorphosis were determined largely on the basis of the morphological descriptions provided by Manion and Stauffer (1970). Anesthetised animals were weighed and measured to the nearest milligram and millimeter respectively, but since it was necessary to extract samples immediately for various other studies, there was insufficient time to measure accurately the lengths of their various morphological structures. After extraction of blood and other samples, the animals were placed in 5% formalin and later measured to the nearest 0.5 mm for snout (preorbital) and disc lengths and eye diameter (see Manion and Stauffer 1970). Since prior treatment damaged the trunk region of the animal, accurate measurement could not be made of the total length of the preserved animal, and measurements of the three structures mentioned above were expressed as percentages of the total length of the live animal. After measurement, the animals were assigned to stages on the basis of the qualitative and photographic assignments of Manion and Stauffer (1970) except that stage IV was restricted to those animals in which the edges of the disc were still apposed (the live animals being unable to attach readily to the side of a tank). We thus follow Hardisty and Potter (1971a) in regarding the period at the beginning of metamorphosis, when massive morphological alterations are taking place in the ammocoete structures (stages I–IV), as the transforming phase, and the subsequent stages when the animal has the superficial appearance of the adult as the macrophthalmia stage. Information on morphological development was based on the examination of over 150 animals at various stages of metamorphosis during 1971.

## Results

An analysis of *P. marinus* caught during 1971 revealed a high degree of synchrony in the developmental changes of metamorphosing individuals. Thus, stages I–IV were found only July 12–26, July 19–August 2, July 29–August 15, and in the latter part of August, respectively. The proportional changes in the disc, snout, and eye measurements showed clear patterns in their development (Fig. 1). Thus, both snout and disc underwent some reduction in early transformation but later increased markedly until just prior to the onset of feeding. On the other hand, the eye increased detectably in size only during the first few weeks of metamorphosis.

The hematocrits of ammocoetes collected between May and December showed great variation (Table 1) but this did not appear to be related to differences in size of animal. On initiation of metamorphosis, hematocrits decreased, the values being similar from stage I to early macrophthalmia (September). In the later macrophthalmia stages (October), the mean hematocrit was significantly higher than that for any other metamorphosing stage. During the parasitic stage, hematocrits of feeding animals held in the laboratory remained at approximately this level. *P. marinus* collected

during the upstream spawning migration had a mean hematocrit higher than for any other post-larval stage.

Polyacrylamide gel electrophoresis clearly showed a major change in the properties of the hemoglobins of *P. marinus* during metamorphosis. Only the fastest moving components in the ammocoete and adult apparently migrated at the same rates (Fig. 1). The patterns, which were the same in the three buffers used, also showed that the overall mobility of the larval components was greater than that of the adults. A very marked difference was observed, both with amido black and O-dianisidine, in the degree of staining of the individual components of both larvae and adults. Thus, in the ammocoete, the bands designated $l_4$ and $l_6$ stained by far the most deeply and the intensity of $l_2$ was greater than those of $l_1$, $l_3$, and $l_5$, which had approximately the same staining characteristics. In the adult, one component ($a_5$) took up more stain than either of the major ammocoete components and far more than any of the other adult hemoglobins. Band $a_4$ was more intense than band $a_6$, which appeared similar to $a_1$. Bands $a_2$ and $a_3$ were little more than traces. No signs of polymorphism could be seen in the three major hemoglobin components of either the 120 ammocoetes or 40 adults investigated. The earliest transforming stages possessed the ammocoete hemoglobin electrophoretic pattern, whereas late November macrophthalmia showed the typical adult situation. A clearly distinguishable mixed pattern was seen in samples from late August and September.

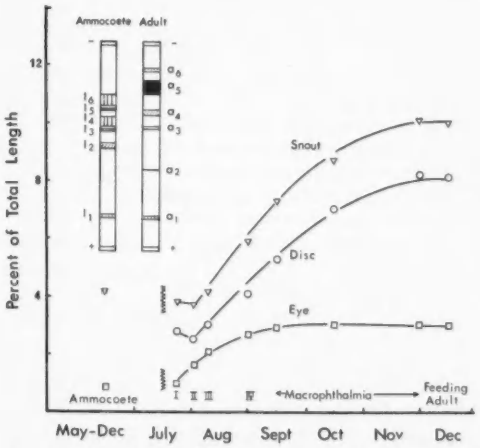


FIG. 1. Lengths of three morphological characters of *P. marinus* (as percentages of total lengths of live animals) during various stages of the life cycle, and a diagrammatic representation of the hemoglobin electrophoretic patterns for ammocoetes and adults. Relative staining intensities of bands: ■ , dark; ▥ , medium dark; ▨ , medium; □ , light; — , trace. Each plotted point represents 20 or more specimens.

Quantitative field information obtained on the timing of downstream migration of macrophthalmia was rather limited. However, examination of the numbers caught suggested that the peak of this movement took place during late October and November and that it mainly occurred during periods of increased water flow. Although some animals remained in the river system during the winter, such a situation was the exception. Thus, extensive sampling between February and May 1969 yielded only six macrophthalmia compared with the vast numbers of metamorphosing lampreys that were found annually between July and October.

Of the macrophthalmia brought into the laboratory from September to the end of October and held at 10 ± 1 C some burrowed, some lay on the substrate, and others remained for long periods attached to the side of the tank. Presentation of teleosts did not result in feeding behavior until

TABLE 1. Hematocrits of *P. marinus* at various stages of the life cycle on basis of samples collected in 1971.

| Date | Stage | No. of animals | Length (*mm*) | | | Hematocrit | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | Range | SD | Mean | Range | SD |
| May–Dec. | Ammocoete | 160 | 122.0 | 70–169 | 23.8 | 28.7 | 16–45 | 6.0 |
| July 12–26 | Transforming I | 4 | 137.0 | 125–148 | 9.1 | 22.2 | 18–31 | 5.9 |
| July 19–Aug. 2 | Transforming II | 6 | 143.2 | 134–164 | 11.4 | 21.5 | 16–28 | 4.3 |
| July 29–Aug. 15 | Transforming III | 8 | 142.0 | 129–147 | 6.3 | 19.5 | 17–23 | 2.3 |
| Aug. 22–30 | Transforming IV | 8 | 139.7 | 131–149 | 6.7 | 20.1 | 13–32 | 6.1 |
| Sept. | Macrophthalmia | 19 | 141.7 | 130–156 | 6.8 | 19.9 | 11–28 | 4.2 |
| Oct. | Macrophthalmia | 12 | 145.2 | 127–160 | 8.7 | 26.4 | 23–34 | 3.1 |
| July–Nov. | Feeding Adult | 5 | 345.0 | 291–410 | 5.0 | 26.0 | 22–30 | 3.4 |
| June–July | Spawning Adult | 19 | 420.0 | 335–493 | 50.0 | 33.0 | 18–41 | 4.6 |

late November but by the middle of December virtually all the animals had attached to fish. Thus, at any time, over 95% of approximately 600 animals were always observed attached and as many as 60–70 lampreys (4–6 g weight) were seen fastened to single fish (*Catostomus commersoni*) of approximately 500 g weight usually resulting in their death within about 4 days.

During December, a number of tanks contained macrophthalmia stages which remained burrowed in the substrate although a few occasionally emerged at night. Fifteen of these animals were induced to leave the substrate and their behavior over the following 48 hr was recorded. Within 5 min, eight had attached to the side of the tank, one had burrowed and the other six were swimming around the tank. A sucker introduced into the tank after 1 hr was attacked by four lampreys within 24 hr and by six within 48 hr. The lampreys remained attached for long periods and the injury caused to the host indicated that they had been feeding. At this time, eight of the other animals were attached to the side of the tank, occasionally detaching and swimming around but only rarely burrowing.

## Discussion

One of the major features emerging from this study of the lamprey population in Shelter Valley Creek is the high degree of synchrony of the various stages of metamorphosis. This is reflected especially by the short time interval in which each of the early transformation stages were present and by the sudden onset in the laboratory of feeding behavior in the majority of animals during late November and early December. This remarkable degree of synchronisation in metamorphosis, after a larval period of several years, has also been observed in other species of lampreys (Leach 1951; Abakumov 1966; Hardisty and Huggins 1970; Potter 1970), suggesting the presence of a very sensitive mechanism responding to environmental changes (Hardisty and Potter 1971a).

The decline in hematocrit values which accompanied metamorphosis at a period when the hemoglobin electrophoretic characteristics were undergoing extensive modification may reflect a rate of replacement of cells containing adult hemoglobin lower than that at which cells containing ammocoete hemoglobins were being removed from the system. The change-over in hemoglobin was completed by the late macrophthalmia stage, coincident with an increase in hematocrit values. Indeed, at this time, hematocrit values were not significantly lower than those of feeding adults. The higher hematocrits for migrating adults than for feeding adults reared in the laboratory may indicate nutritional inadequacies or, more probably, an adaptation to increase the oxygen carrying capacity of the blood during the spawning migration. Indeed Ivanova Berg and Sololova (1959) have shown that the high values at the commencement of migration of *L. fluviatilis* suffer a gradual but marked decline through to spawning. However, it should be noted that *L fluviatilis* and the majority of other species (Hardisty and Potter 1971a) have a spawning migration lasting considerably longer than that of *P. marinus*.

The mean hematocrit value in the migrating adults (33.0) is identical to that found by Thorson (1959) for this species. Manwell (1963) measured hematocrits as high as 40 in the migrating adults, comparable to the highest hematocrit (41) in the present study. A lower value of 23.5 was found by Kisch (1951) but was based on only one specimen. A mean hematocrit value and SE of $24.7 \pm 1.9$ obtained for ammocoetes of *Ichthyomyzon hubbsi* (Potter et al. 1970) was not significantly different from that found for ammocoetes of *P. marinus* in this study.

The qualitative characteristics of the hemoglobin electrophoretic patterns of the larval and adult stages from Shelter Valley Creek corresponded closely with those obtained by starch gel techniques for other landlocked populations of *P. marinus* from different areas (Manwell 1963; Uthe et al. 1966; Uthe and Tsuyuki 1967). Thus, in all these studies, the three darkest bands of the ammocoete exhibit the same relative staining intensities, and the middle of the three main adult components is always predominant. However, in the ammocoete, polyacrylamide gel electrophoresis revealed the presence of three, less prominent components, while in the adult electrophoregrams the other three components, all fast migrating and lightly staining may correspond to the minor hemoglobins of anadromous *P. marinus*, which continuous flow electrophoresis has shown to possess at least five and often six hemoglobins (Rumen and Love 1963).

Our study clearly demonstrated that there is no correspondence in electrophoretic mobility between the major hemoglobin components of the ammocoete and adult. The change in chemical properties of hemoglobins at metamorphosis is associated with an alteration in their oxygen equilibria characteristics (Manwell 1963). Furthermore, work on amphibian metamorphosis has shown that although in *Rana catesbeiana* there is a difference in the morphology of erythrocytes containing larval and adult hemoglobin (Moss and Ingram 1968), the change-over, at least in *Xenopus laevis*, appears to occur within one cell line and does not result from two populations exclusively synthesizing each of

the two sets of hemoglobins (Jurd and Maclean 1970).

As in most other electrophoretic studies on lamprey proteins, no evidence was found of polymorphism in the major components of either ammocoete or adult. This feature, implying homogeneity, is perhaps remarkable in view of the antiquity of the group, a feature at least partly attributable to a marked ability to modify the life cycle. Thus, an anadromous parasitic species, such as *P. marinus*, has given rise to landlocked populations, and several other anadromous and freshwater parasitic lampreys have apparently been ancestral to species which have eliminated the parasitic phase of their life cycle (for reviews see Hardisty and Potter 1971b; Hubbs and Potter 1971).

During metamorphosis, the animals show a tendency to move into areas where the water flow is faster and the substrate consists of larger particle size. This movement may result in the animals being exposed to an increase in dissolved oxygen, which in the initial phase of metamorphosis would be advantageous as their respiratory apparatus is undergoing such extensive changes (Damas 1935) that they may have to rely heavily on cutaneous respiration. Since many animals attached to the side walls of tanks during September, this change must be accomplished rapidly, probably within 4 weeks. In fact, selection pressures to keep this period at the shortest possible duration are probably very high as the animals would at this time be susceptible to any major environmental changes. In the later stages of metamorphosis, the higher oxygen concentrations would also be advantageous since adult hemoglobins now predominate in the blood and these have been shown to have a lower affinity for oxygen than those of the ammocoete (Manwell 1963).

Metamorphosis can justifiably be considered as completed when the animal has concluded its downstream migration and commenced feeding. However, as pointed out, such a criterion represents a maximum duration and can be rather misleading as animals sometimes remain in the river for varying periods even though they have apparently reached similar developmental stages.

From the data collected for *P. marinus* an attempt can be made to produce a picture of the timing of the various phases of metamorphosis in Shelter Valley Creek. While the first external changes were observable in mid-July (at least in 1971), a time very similar to that recorded for other landlocked populations of *P. marinus* (Applegate 1950; Manion and Stauffer 1970), the termination of metamorphosis must be inferred from data on downstream migration and on commencement of feeding in the laboratory. Since virtually all the animals brought to the laboratory between September and November fed before the end of the year, the necessary structural and physiological changes essential for the parasitic phase of the life cycle had been completed. Although the slightly higher temperatures in the laboratory may have accelerated development, the fact that downstream migration very largely took place in late October and November indicates that the animals were probably at least in a stage closely approaching that of functional feeding adults. Assuming this to be the case, the time of completion of metamorphosis can be regarded as late October to the end of November. Such an estimate is compatible with the fact that the change-over in hemoglobins was apparently complete at the end of this period and snout and disc development reached a "plateau" just before the early feeding stages. This emphasizes the limitations of the time of emergence as a criterion for completion of metamorphosis, an especially dubious one under artificial conditions in view of the variability in their behavior in the laboratory after being removed from their natural environment. In this context, it is worth noting that one of us (I.C.P.) has also observed vastly different times of emergence in laboratory aquaria in both *Lampetra fluviatilis* and *Mordacia mordax*.

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ABAKUMOV, V. A. 1966. (Systematics and ecology of the Ukrainian lamprey (*Lampetra mariae* Berg)). Vop. Ikhtiol. 4: 423–432. (In Russian)

ADINOLFI, M., AND G. CHIEFFI. 1958. Larval and adult haemoglobins of the cyclostome *Petromyzon planeri*. Nature (London) 182: 730.

ADINOLFI, M., G. CHIEFFI, AND M. SINISCALCO. 1959. Haemoglobin pattern of the cyclostome *Petromyzon planeri*. Nature (London) 184: 1325–1326.

APPLEGATE, V. C. 1950. Natural history of the sea lamprey, *Petromyzon marinus* in Michigan. U.S. Fish Wildl. Serv. Spec. sci. Rep. Fish. 55: 1–237.

APPLEGATE, V. C., AND C. L. BRYNILDSON. 1952. Downstream movement of recently transformed sea lampreys, *Petromyzon marinus*, in the Carp Lake River, Michigan. Trans. Amer. Fish. Soc. 81: 275–290.

BALABAI, P. P. 1948. (The time needed for metamorphosis in *Lampetra mariae* Berg). Tr. Inst. Zool. Kyyiv 1: 29–38. (In Ukrainian)

DAMAS, H. 1935. Contribution a l'étude de la métamorphose de la tête de la lamproie. Arch. Biol. Paris 46: 171–227.

GAGE, S. H. 1928. The lampreys of New York State — life history and economics. Biological survey of the Oswego River System. Rep. N.Y. State Conserv. Dep. (Suppl.) 17: 158–191.

HARDISTY, M. W., AND R. J. HUGGINS. 1970. Larval growth in the river lamprey *Lampetra fluviatilis* L. J. Zool. (London) 161: 549–559.

HARDISTY, M. W., AND I. C. POTTER. 1971a. The general biology of adult lampreys, p. 127–206. *In* M. W. Hardisty and I. C. Potter [ed.] The biology of lampreys. Academic Press, Inc., London, England.

1971b. Paired species, p. 249–277. *In* M. W. Hardisty and I. C. Potter [ed.] The biology of lampreys. Academic Press, Inc., London, England.

HARDISTY, M. W., I. C. POTTER, AND R. STURGE. 1970. A comparison of the metamorphosing and macrophthalmia stages of the lampreys *Lampetra fluviatilis* and *L. planeri*. J. Zool. (London) 162: 383–400.

HUBBS, C. L., AND I. C. POTTER. 1971. Distribution, phylogeny and taxonomy, p. 1–65. *In* M. W. Hardisty and I. C. Potter [ed.] The biology of lampreys. Academic Press, Inc., London, England.

IVANOVA BERG, M. M., AND M. M. SOLOLOVA. 1959. (Seasonal changes in the blood composition of *Lampetra fluviatilis* L.) Vop. Ikhtiol. 13: 156–162. (In Russian)

JURD, R. D., AND N. MACLEAN. 1970. An immunofluorescent study of the haemoglobins in metamorphosing *Xenopus laevis*. J. Embryol. Exp. Morphol. 23: 299–309.

KISCH, B. 1951. Erythrocytes in fishes. Exp. Med. Surg. 9: 125–137.

LEACH, W. J. 1951. The hypophysis of lampreys in relation to the nasal apparatus. J. Morphol. 89: 217–256.

LI, S. I., AND A. RIGGS. 1970. The amino acid sequence of Haemoglobin V from the lamprey, *Petromyzon marinus*. J. Biol. Chem. 245: 6149–6169.

MANION, P. J., AND T. M. STAUFFER. 1970. Metamorphosis of the landlocked sea lamprey, *Petromyzon marinus*. J. Fish. Res. Bd. Canada 27: 1735–1746.

MANWELL, C. 1963. The blood proteins of Cyclostomes. A study in phylogenetic and ontogenetic biochemistry, p. 372–455. *In* A. Brodal and R. Fange [ed.] The biology of Myxine. Universitetsforlaget Oslo.

MOSS, B., AND V. M. INGRAM. 1968. Haemoglobin synthesis during amphibian metamorphosis. II. Synthesis of adult haemoglobin following thyroxine administration. J. Mol. Biol. 32: 493–504.

OWEN, J. A., AND H. SMITH. 1961. Detection of ceruloplasmin after zone electrophoresis. Clin. Chim. Acta 6: 441–444.

POTTER, I. C. 1970. The life cycles and ecology of Australian lampreys of the genus *Mordacia*. J. Zool. (London) 161: 487–511.

POTTER, I. C., B. J. HILL, AND S. GENTLEMAN. 1970. Survival and behavior of ammocoetes at low oxygen tensions. J. Exp. Biol. 53: 59–73.

POTTER, I. C., AND P. I. NICOL. 1968. Electrophoretic studies on the haemoglobins of Australian lampreys. Aust. J. Exp. Biol. Med. Sci. 46: 639–641.

RALL, D. P., P. SCHWAB, AND G. C. ZUBROD. 1961. Alteration of plasma proteins at metamorphosis in the lamprey *Petromyzon marinus*. Science 133: 279–280.

RUMEN, N. M., AND W. E. LOVE. 1963. The six haemoglobins of the sea lamprey (*Petromyzon marinus*). Arch. Biochem. Biophys. 103: 23–25.

SMITHIES, O. 1959. An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. Biochem. J. 71: 585–587.

THORSON, T. B. 1959. Partioning of body in sea lamprey. Science 130: 99–100.

UTHE, J. F., E. ROBERTS, L. W. CLARKE, AND H. TSUYUKI. 1966. Comparative electrophoregrams of representatives of the families Petromyzonitidae, Esochidae, Centrarchidae and Percidae. J. Fish. Res. Bd. Canada 23: 1663–1671.

UTHE, J. F., AND H. TSYUKI. 1967. Comparative zone electrophoregrams of muscle myogens and blood proteins of adult and ammocoete lamprey. J. Fish. Res. Bd. Canada 24: 1269–1273.

VLADYKOV, V. D. 1949. Quebec lampreys (Petromyzondiae). I. List of species and their economical importance. Contr. Dep. Pech. Que. 26: 1–67.

WIGLEY, R. L. 1959. Life history of the sea lamprey of Cayuga Lake, New York. Fish. Bull. U.S. 59: 561–617.

# A Survey of the Total Cadmium Content of 406 Fish from 49 New York State Fresh Waters

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Fish from New York State fresh waters were surveyed for total cadmium. The majority of samples contained 20 ppb or below. The remainder showed concentrations up to 100 ppb with only few above this concentration. Fishes from central New York waters rarely contained cadmium greater than 20 ppb. Fish from Adirondack waters contained cadmium above 20 ppb most consistently. These higher concentrations may be related to generally higher background cadmium levels in this Adirondack area where many metallic ore deposits are located with which cadmium is typically associated. Cadmium accumulation only occasionally appeared species-dependent. No relation was obvious between total residues of the metal and size or sex of fish or age of lake trout. The cadmium concentrations observed are comparable to those commonly present in many other foods.

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Nous avons fait un relevé de la teneur en Cd total des poissons habitant les eaux douces de l'Etat de New York. La plupart des échantillons contiennent 20 ppb de Cd ou moins. Les autres ont une teneur atteignant 100 ppb, quelques échantillons seulement dépassant cette concentration. Les poissons provenant du centre de l'Etat contiennent rarement plus de 20 ppb de Cd. Ceux de la région des Adirondack dépassent le plus souvent ce niveau. Ces hautes teneurs en Cd peuvent être attribuables aux niveaux élevés de Cd dans une région riche en minérais avec lesquels le Cd est typiquement associé. L'accumulation de Cd ne semble qu'occasionnellement liée à l'espèce. Il n'y a pas de relation évidente entre les résidus totaux du métal et la taille ou le sexe des poissons, ou l'âge du touladi. Les teneurs en Cd observées sont comparables à celles qu'on rencontre communément dans beaucoup d'autres aliments.

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SINCE the discovery of ubiquitous environmental pollution by mercury in North America, especially in fish, the suspicion has been publicized that other toxic metals may similarly be widespread contaminants. Of the list of possible metals, much concern has been expressed about cadmium since it concentrates in the liver, kidney, and certain other organs of animals and humans (Truhaut and Boudene 1954). Cadmium has been implicated as a possible cause of hypertension (Schroeder and Vinton 1962; Schroeder 1964; 1965; Schroeder et al. 1966; 1968; Carroll 1966), kidney ailments

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(Piscator 1962; Axelsson and Piscator 1966), anosmia and testicular atrophy (Gunn et al. 1963), and shortened life span (Schroeder et al. 1971). The toxic effects and biopathology of cadmium in animals and humans have been reviewed (Flick et al. 1971; Vigliani 1969).

Sources of cadmium in the environment are many (Dunphy 1967; Athanassiadis 1969; Largerwerff and Specht 1970). Extensive cadmium pollution of water from industrial wastes has been reported (Perlmutter et al. 1963). Little data is available on the cadmium content of fish, but it concentrates in the liver (Lucas et al. 1970). In sufficient concentrations, cadmium may exert its toxic effects on fish by coagulating external gill mucus, causing anoxia and altered salt balance and secretion of waste products (Carpenter 1927, 1930). Heavy metals such as cadmium also effectively poison various fish liver enzymes (Jackim et al. 1970). For fish, the lethal concentration of cadmium in water ranges from 0.01 to 10 ppm, depending on many factors (Schweiger 1957). Current theories concerning modes of toxic action of metals to fish have been described (Water Quality Criteria 1968).

An average concentration of 94 ppb of cadmium has been reported for 19 fish of 3 species from the Great Lakes (Lucas et al. 1970) and shellfish appear to be particularly efficient accumulators of the metal (Pringle et al. 1968). The importance of determining the concentration of heavy metals in fish has been declared by federal agencies (Pollution: Hazards from Metals 1970). In the work reported, a survey of the total cadmium content of 406 fish from 49 New York State waters has been made.

## Experimental Methods

In cooperation with the New York State Department of Environmental Conservation, fish were made available as part of the department's 1969 annual, statewide fish sampling program. All fish were netted and records were kept of species, sex, length, and weight. The fish were decapitated and eviscerated. The remainder was minced in a food chopper, thoroughly mixed, and frozen in polyethylene bags prior to analysis. Ten grams of each fish was dry-ashed at 485 C, using the procedure of Evans and Bandemer (1954) except that no magnesium nitrate was added. The ash was dissolved in 0.8 N HCL and analyzed by atomic absorption analysis using the Perkin-Elmer Model 303 atomic absorption spectrophotometer with tantalum boat accessory. Throughout the study a standard cadmium solution was analyzed after each of two samples to maintain accurate calibration. Recoveries of 0.025–0.15 ppm of cadmium added to fish prior to drying and combustion were in the range of 75–85%, after subtraction of their initial cadmium concentration as determined by analysis. The sensitivity of the method was 10 ppb of cadmium in fish on a fresh weight basis.

## Results and Discussion

Table 1 lists alphabetically the species of fish collected. Locations of waters from which fish were netted are shown on the New York State map (Fig. 1). The results of analysis of the fish for cadmium are presented (Table 2). The standard error was computed only when three or more fish of a given species were analyzed. The concentrations of cadmium listed are in parts per billion on a fresh weight basis and are not corrected for percent recovery.

Of the total number of fish analyzed, 68.5% contained 20 ppb or less of cadmium. From waters in which at least five fish were netted, levels of cadmium above 20 ppb were found in fish from 12 lakes — Blue Mountain, Butterfield, Champlain, Erie, Fourth, George, Hemlock, Long, Ontario, Placid, Pleasant, and Raquette — 2 rivers — Hudson and St. Lawrence — and Cattaraugus Creek. Cadmium concentrations above 100 ppb were found in only one fish from each of lakes Erie, Fourth, and Piseco, and in five fish from the Hudson River. Fifty percent of the fish from Adirondack lakes

TABLE 1. Common and scientific names of fish analyzed in this study.

| Common name | Scientific name |
|---|---|
| Black crappie | *Pomoxis nigromaculatus* |
| Bowfin | *Amia calva* |
| Brook trout (speckled trout) | *Salvelinus fontinalis* |
| Brown trout | *Salmo trutta* |
| Bullhead | *Ictalurus* sp. |
| Burbot | *Lota lota* |
| Carp | *Cyprinus carpio* |
| Catfish | *Ictalurus* sp. |
| Chain pickerel | *Esox niger* |
| Cisco | *Coregonus artedii* |
| Coho salmon | *Oncorhynchus kisutch* |
| Freshwater drum | *Aplodinotus grunniens* |
| Gizzard shad | *Dorosoma cepedianum* |
| Goldfish | *Carassius auratus* |
| Lake trout | *Salvelinus namaycush* |
| Lake whitefish | *Coregonus clupeaformis* |
| Largemouth bass | *Micropterus salmoides* |
| Muskelunge | *Esox masquinongy* |
| Northern pike | *Esox lucius* |
| Perch | *Perca* sp |
| Rainbow trout | *Salmo gairdneri* |
| Rock bass | *Ambloplites rupestris* |
| Smallmouth bass | *Micropterus dolomieui* |
| Splake (brook × lake trout) | |
| Striped bass | *Morone saxatilis* |
| Sturgeon | *Acipenser* sp. |
| Walleye pike | *Stizostedion v. vitreum* |
| White bass (Silver bass) | *Roccus chrysops* |
| White sucker (Sucker) | *Catostomus commersoni* |

FIG. 1. New York State map showing approximate locations of waters.

showed cadmium levels above 20 ppb, while only 24% of the fish from all other parts of the State were above this level. The range of cadmium concentrations found in fish from New York waters (10–170 ppb) thus appears comparable to the range (47–200 ppb) reported for Great Lakes fish (Lucas and Edgington 1970).

Correlation between fish weight and cadmium concentrations was not significant for two species having an adequate sample size for comparisons. These samples were coho salmon from Lake Ontario and freshwater drum from Lake Erie and had correlation coefficients of −0.25 and 0.036, respectively; neither is significant. Inspection of original data for other species supports these analyses. In certain instances particular fish species seemed to contain higher cadmium levels. This was true of silver bass from Lake Erie, whitefish from Raquette Lake, goldfish from the Hudson River, and sturgeon from the Hudson and St. Lawrence rivers.

Total cadmium in a series of lake trout sampled in 1970 from Cayuga Lake and ranging in age from 1 to 12 years is shown below. The age was known, since the fish are annually stocked as yearlings and distinctly marked as to year-class. From this limited data, cadmium does not appear to be age-dependent in lake trout ($r = -0.17$, not significant), as follows:

| Age (*years*) | Cadmium (*ppb*) |
|---|---|
| 1 | 15 |
| 2 | 18 |
| 3 | 29 |
| 4 | 25 |
| 5 | 18 |
| 6 | 25 |
| 7 | 12 |
| 8 | 15 |
| 9 | 17 |
| 11 | 18 |
| 12 | 21 |

Some general remarks may be made regarding the results. Cadmium is often associated with other minerals such as zinc, lead-zinc, and lead-copper-zinc

TABLE 2. Residues of total cadmium in fish from New York State waters in 1969 (italics refer to locations in Fig. 1. Numbers of fish in parentheses).

| Species | Length (*cm*) | Weight (*kg*) | Mean residue (*ppb*) and SE |
|---|---|---|---|
| Blue Mountain Lake *1* | | | |
| Bullhead–catfish (3) | 27.9–28.9 | 0.377–0.379 | 27.3(6.06) |
| Rainbow trout (2) | 33.0–33.8 | 0.173–0.393 | 24.0 |
| Smallmouth bass (1) | 28.2 | 0.275 | 22.0 |
| Butterfield Lake *2* | | | |
| Bowfin (1) | 55.9 | 1.702 | 16.0 |
| Bullhead–catfish (3) | 32.0–33.0 | 0.669–0.685 | 18.7(0.32) |
| Largemouth bass (3) | 33.0–34.8 | 0.573–0.657 | 20.0(1.15) |
| Northern pike (4) | – | – | 44.7(13.60) |
| Rock bass (2) | 22.2–22.9 | 0.263–0.277 | 36.0 |
| Smallmouth bass (2) | – | – | 21.5 |
| Sucker (1) | 38.6 | 0.916 | 20.0 |
| Walleye pike (3) | 47.3–55.9 | 1.071–1.753 | 16.3(2.85) |
| Canadice Lake *3* | | | |
| Bullhead–catfish (1) | 35.0 | 0.574 | 18.0 |
| Lake trout (1) | 47.8 | 1.246 | <10 |
| Pickerel (2) | 40.7–46.0 | 0.471–0.699 | 11.5 |
| Rainbow trout (2) | 27.4–29.2 | 0.243–0.284 | 15.5 |
| Rock bass (1) | 21.8 | 0.248 | 14.0 |
| Smallmouth bass (5) | 28.4–36.1 | 0.378–0.751 | 13.0(1.48) |
| Canandaigua Lake *4* | | | |
| Brown trout (1) | 56.7 | 2.595 | 11.0 |
| Lake trout (3) | 44.4–55.2 | 0.722–1.821 | <10(0) |
| Largemouth bass (1) | 28.7 | 0.370 | <10 |
| Smallmouth bass (1) | 28.7 | 0.359 | 19.0 |
| Conesus Lake *5* | | | |
| Black crappie (1) | – | – | 12.0 |
| Caroga Lake *6* | | | |
| Smallmouth bass (1) | 40.9 | 1.295 | 21.0 |
| Cattaraugus Creek *7* | | | |
| Coho salmon (9) | 37.9–64.0 | 0.588–3.04 | 24.2(7.27) |
| Cayuga Lake *8* | | | |
| Largemouth bass (1) | 15.25 | – | <10 |
| Pickerel (chain) (3) | – | – | <10(0) |
| Chenango River *9* | | | |
| Smallmouth bass (3) | 22.2–28.6 | 0.168–0.295 | 13.7(1.20) |
| Chittenango Creek *10* | | | |
| Brown trout (3) | 23.4–43.2 | 0.151–1.072 | 20.7(8.74) |
| Cohocton River *11* | | | |
| Brown trout (3) | 25.4–27.6 | 0.194–0.256 | <10(0) |
| 8th Lake *12* | | | |
| Rainbow trout (1) | – | – | 19.0 |
| Lake Erie *13* | | | |
| Burbot (1) | 58.4 | 1.980 | <10 |
| Coho salmon (6) | 41.3–57.5 | 0.665–2.671 | 19.0(4.84) |
| Lake Erie *13* | | | |
| Freshwater drum (19) | 29.2–50.8 | 0.270–1.750 | 28.9(8.21) |
| Gizzard shad | Composite of 31 fish | | 72.0 |
| Lake trout (1) | – | – | <10 |
| Rock bass (1) | 27.3 | 0.358 | 20.0 |
| Silver bass (8) | 34.9–40.0 | 0.425–0.840 | 28.0(3.88) |
| Smallmouth bass (10) | 33.0–45.7 | 0.360–1.300 | 15.9(1.04) |
| Sucker (11) | 30.5–51.5 | 0.334–1.530 | 23.1(4.65) |

(*Continued*)

TABLE 2. Residues of total cadmium in fish from New York State waters in 1969 (italics refer to locations in Fig. 1. Numbers of fish in parentheses). — (*Continued*)

| Species | Length (*cm*) | Weight (*kg*) | Mean residue (*ppb*) and SE |
|---|---|---|---|
| Walleye pike (8) | 62.3–73.7 | 2.920–5.070 | 16.0(1.92) |
| Yellow perch (10) | 27.9–36.2 | 0.450–0.735 | 16.4(1.08) |
| Fern Lake *14* | | | |
| Rock bass (1) | 26.2 | 0.426 | 21.0 |
| Forked Lake *15* | | | |
| Lake trout (1) | 53.3 | 0.854 | 54.0 |
| Smallmouth bass (1) | 37.1 | 0.950 | 26.0 |
| Fourth Lake *16* | | | |
| Lake trout (3) | 37.8–55.9 | 0.497–1.872 | 80.7(30.12) |
| Sucker (2) | 29.2–30.2 | 0.301–0.336 | 18.5 |
| Genessee River *17* | | | |
| Smallmouth bass (1) | – | – | 14.0 |
| Hemlock Lake *18* | | | |
| Bullhead–catfish (3) | 28.2–36.1 | 0.279–0.739 | 13.3(1.76) |
| Chain pickerel (3) | 31.7–50.8 | 0.229–1.245 | 17.7(4.10) |
| Lake trout (3) | 67.5–77.8 | 3.987–4.580 | <10 (0) |
| Largemouth bass (1) | 26.2 | 0.344 | <10 |
| Rainbow trout (1) | 38.1 | 0.688 | 13.0 |
| Hudson River *19* | | | |
| Atlantic sturgeon (4) | 46.0–98.0 | 0.410–5.076 | 91.5(21.72) |
| Goldfish (3) | – | – | 142.7(21.46) |
| Short Nose sturgeon (1) | 47.3 | 0.636 | |
| Striped bass (6) | 40.6–57.6 | 0.780–2.256 | 12.2(1.11) |
| Indian Lake *20* | | | |
| Northern pike (2) | 64.3–73.0 | 1.433–2.735 | 18.0 |
| Whitefish (1) | 43.2 | 0.789 | 24.0 |
| Lake Champlain *21* | | | |
| Channel catfish (6) | 47.8–76.1 | 1.130–6.200 | 23.2(12.97) |
| Freshwater drum (3) | 31.7–48.8 | 0.306–1.165 | 14.0(0.58) |
| Walleye pike (3) | 40.2–64.8 | 0.566–3.070 | <10(0) |
| Lake George *22* | | | |
| Black crappie (3) | 20.3–31.2 | 0.109–0.584 | 14.7(1.20) |
| Brown bullhead (3) | 32.8–33.3 | 0.418–0.686 | 17.7(4.63) |
| Bullhead–catfish (1) | 30.0 | 0.469 | 92.0 |
| Chain pickerel (1) | 35.6 | 0.258 | 31.0 |
| Lake trout (4) | 68.8–71.0 | 3.125–3.570 | 16.0(5.35) |
| Largemouth bass (3) | 36.3–41.1 | 0.743–0.973 | 16.3(2.91) |
| Northern pike (5) | 59.5–75.0 | 1.780–3.180 | 13.8(1.46) |
| Rainbow trout (4) | 50.0–58.6 | 1.690–2.440 | 14.8(4.75) |
| Smallmouth bass (1) | 42.4 | 1.235 | <10 |
| White sucker (3) | 46.3–53.0 | 0.872–1.139 | 17.0(3.06) |
| Lake Ontario *23* | | | |
| Black crappie (1) | – | – | 15.0 |
| Carp (1) | 27.4 | 0.419 | <10 |
| Coho salmon (21) | 50.0–66.0 | 0.965–3.780 | 13.5(0.84) |
| Rock bass (4) | 22.8–25.5 | 0.268–0.436 | 17.2(3.30) |
| Smallmouth bass (10) | 27.4–37.6 | 0.258–1.014 | 13.6(1.33) |
| Splake (1) | 34.8 | 0.523 | <10 |
| Sucker (2) | 39.1 | 0.657–0.703 | 23.5 |
| White bass (1) | 25.5 | 0.272 | <10 |
| Largemouth bass (1) | – | – | 13.0 |
| Lake Placid *24* | | | |
| Brook trout (1) | 30.5 | 0.360 | 23.0 |
| Lake trout (2) | 31.7–41.5 | 0.299–0.726 | 28.5 |

(*Continued*)

TABLE 2. Residues of total cadmium in fish from New York State waters in 1969 (italics refer to locations in Fig. 1. Numbers of fish in parentheses). — (*Continued*)

| Species | Length (*cm*) | Weight (*kg*) | Mean residue (*ppb*) and SE |
|---|---|---|---|
| Northern pike (3) | 57.4–76.6 | 1.408–3.870 | 16.3(2.85) |
| Rainbow trout (3) | 25.4–42.1 | 0.225–0.779 | 17.0(1.15) |
| Smallmouth bass (2) | 29.2–46.3 | 0.336–1.420 | 31.0 |
| White sucker (2) | 30.5–43.7 | 0.417–1.440 | 16.0 |
| Yellow perch (1) | 31.7 | 0.435 | 30.0 |
| Lake Pleasant *25* | | | |
| Brown bullhead (2) | 34.3–34.8 | 0.673–0.681 | 26.0 |
| Bullhead–catfish (1) | 25.5 | 0.309 | 18.0 |
| Chain pickerel (3) | 35.0–39.2 | 0.255–0.405 | 22.0(2.65) |
| Largemouth bass (1) | 35.0 | 0.622 | 21.0 |
| Rainbow trout (1) | 42.7 | 1.068 | 27.0 |
| Rock bass (2) | 25.4–26.4 | 0.325–0.461 | 63.5 |
| Smallmouth bass (3) | 32.7–49.3 | 0.484–1.775 | 39.3(16.37) |
| Whitefish (2) | 53.8–61.0 | 2.035–2.153 | 60.0 |
| Yellow perch (2) | 27.4–30.0 | 0.335–0.438 | 51.0 |
| Little Green Pond *26* | | | |
| Rainbow trout (3) | 32.0–38.6 | 0.370–0.608 | 13.7(2.33) |
| Whitefish (3) | 50.8–57.1 | 1.805–2.050 | 17.0(1.15) |
| Little York Lake *27* | | | |
| Brown trout (1) | 34.3 | 0.456 | <10 |
| Long Lake *28* | | | |
| Largemouth bass (1) | 43.2 | 1.128 | 20.0 |
| Northern pike (3) | 53.8–71.4 | 1.335–2.305 | 38.0(16.50) |
| Smallmouth bass (2) | 35.6–40.9 | 0.672–0.767 | 23.0 |
| Loon Lake *29* | | | |
| Smallmouth bass (1) | – | – | 19.0 |
| Walleye pike (3) | – | 0.187–0.232 | 28.7(12.88) |
| Oneida Lake *30* | | | |
| Walleye pike (2) | 48.3–59.6 | 1.030–2.060 | <10 |
| Onondaga Creek *31* | | | |
| Brown trout (2) | 29.5–32.2 | 0.314–0.386 | 18.5 |
| Otsego Lake *32* | | | |
| Cisco (3) | 25.9–36.1 | 0.161–0.488 | 12.3(0.88) |
| Lake trout (3) | – | – | 13.7(1.20) |
| Whitefish (3) | – | – | 15.0(1.00) |
| Pepacton Reservoir *33* | | | |
| Brown trout (3) | 41.2–64.8 | 0.953–3.115 | 10.3(0.33) |
| Piseco Lake *34* | | | |
| Brown bullhead (1) | 31.7 | 0.425 | 40.0 |
| Whitefish (2) | 31.7–38.1 | 0.295–0.431 | 115.0 |
| Raquette Lake *35* | | | |
| Lake trout (1) | – | – | 23.0 |
| Smallmouth bass (3) | 27.4–28.9 | 0.251–0.287 | 18.3(3.18) |
| Whitefish (6) | 31.7–35.6 | 0.337–0.497 | 33.5(3.74) |
| Rushford Lake *36* | | | |
| Lake trout (1) | 75.2 | 4.195 | <10 |
| Saranac Lake *37* | | | |
| Smallmouth bass (1) | 33.2 | 0.684 | 29.0 |
| Salmon River *38* | | | |
| Coho salmon (18) | 30.5–63.0 | 0.280–2.072 | 11.0(0.35) |
| Saratoga Lake *39* | | | |
| Walleye pike (1) | – | 0.357 | 42.0 |
| Schroon Lake *40* | | | |
| Lake trout (1) | – | – | 37.0 |

(*Continued*)

TABLE 2. Residues of total cadmium in fish from New York State waters in 1969 (italics refer to locations in Fig. 1. Numbers of fish in parentheses). — (*Concluded*)

| Species | Length (*cm*) | Weight (*kg*) | Mean residue (*ppb*) and SE |
|---|---|---|---|
| Skaneateles Lake *41* | | | |
| Rainbow trout (2) | 38.1–50.8 | 0.644–1.235 | 16.0 |
| Spring Brook *42* | | | |
| Coho salmon (3) | 54.4–61.5 | 1.710–2.680 | 16.3(4.91) |
| St. Lawrence River *43* | | | |
| Brown bullhead (1) | – | – | 23.0 |
| Muskie (1) | 77.5 | 3.832 | 30.0 |
| Smallmouth bass (6) | 26.8–30.0 | 0.263–0.398 | 24.8(8.67) |
| Sturgeon (11) | 89.6–103.5 | 3.630–8.200 | 26.2(3.45) |
| Walleye pike (4) | 45.3–65.5 | 1.060–3.527 | 14.5(1.32) |
| Susquehanna River *44* | | | |
| Walleye pike (1) | – | – | 64.0 |
| Yellow perch (2) | 24.8–27.9 | 0.166–0.268 | 51.0 |
| Trout Lake *45* | | | |
| Chain pickerel (3) | 46.3–62.0 | 0.785–1.570 | 14.3(1.45) |
| Smallmouth bass (1) | 43.7 | 1.197 | 17.0 |
| Upper Saranac Lake *46* | | | |
| Largemouth bass (2) | 31.2–34.8 | 0.432–0.904 | 14.0 |
| Utowana Lake *47* | | | |
| Smallmouth bass (2) | 42.5–42.9 | 0.949–1.030 | 12.5 |
| Rainbow trout (2) | 41.5–43.0 | 0.775–0.844 | 17.0 |
| Waneta Lake *48* | | | |
| Muskie (1) | – | – | 14.0 |
| West Canada Lake *49* | | | |
| Lake trout (2) | 38.6–53.3 | 0.426–1.268 | 30.0 |

ores (Athanassiadis 1969). Many metal deposits and mines containing lead and certain others bearing zinc and copper ores occur in New York's Adirondack region (Luedke et al. 1959). Owing to the wilderness of large portions of the region, other unknown deposits may exist. Secondary serpentine minerals, which also abound in the area, may thus be high in cadmium. The preponderance of fish from Adirondack lakes showing cadmium levels relatively higher than fish from, for instance, central New York lakes may, therefore, reflect a higher regional background of the metal. Little data is available on the concentration of cadmium in New York State waters. Cadmium levels ranging from 4 to 12 ppb were reported for water from Lake Erie at Buffalo during the period from 1962 to 1967 (Kopp and Kroner 1970).

Many external factors influence the toxicity and absorption of metals by fish. These include the nature and concentration of the metal, its valence, the form of the metal in water (e.g. as a soluble chelate), the associated anion, the antagonistic or synergistic effect of other cations, *p*H, time of exposure, volume of water, whether stationary or moving water, temperature, dissolved oxygen, and the nature (feeding habits, etc.) and condition of the fish (Doudoroff and Katz 1953).

Schroeder et al. (1967) has determined cadmium in an exhaustive list of food and other environmental samples. The range of cadmium concentrations found in our study must be regarded as comparable to those found in fruits and vegetables and low compared to levels commonly found in seafood, meats, dairy products, cereals and grains, oils, fats, condiments, and many other common foods (Pringle et al. 1968; Murphy et al. 1970; Murthy et al. 1971; Malin 1971). The cadmium concentrations of the fish reported here would not appear to constitute a hazard for consumers. In this regard, zinc is known to exert a pronounced protective effect on the toxicity of cadmium in animals and humans (Schroeder et al. 1967). It is, therefore, interesting to note that the total zinc concentration of fish sampled from 23 New York State waters in 1970 averaged 6.9 with a range of .9–38.5 ppm. These comparatively high zinc levels may, therefore, offer a further safeguard against any remotely possible toxic effects of cadmium.

ANON. 1970. Pollution: hazards from metals. Chem. Eng. News 9 (7): 12–13.

ATHANASSIADIS, Y. C. 1969. Air pollution aspects of cadmium and its compounds. Clearinghouse for Federal Scientific and Technical Information, Springfield, Va. 73 p.

AXELSSON, B., AND M. PISCATOR. 1966. Renal damage after prolonged exposure to cadmium. Arch. Environ. Health 12: 360–373.

CARPENTER, K. E. 1927. The lethal action of soluble metallic salts on fishes. Brit. J. Exp. Biol. 4: 378–390.

1930. Further researches on the action of metallic salts on fishes. J. Exp. Zool. 56: 407–422.

CARROLL, R. E. 1966. The relationship of cadmium in the air to cardiovascular disease death rates. J. Amer. Med. Ass. 198: 267–269.

DOUDOROFF, P., AND KATZ. 1953. Critical review of literature on the toxicity of industrial wastes and their compounds to fish. II. The metals as salts. Sewage Ind. Wastes 25: 802–839.

DUNPHY, B. 1967. Acute occupational cadmium poisoning a critical review of the literature. J. Occup. Med. 9: 22–26.

EVANS, R. J., AND S. L. BANDEMER. 1954. Determination of arsenic in biological materials. Anal. Chem. 26: 595–598.

FLICK, D. F., H. F. KRAYBILL, AND J. M. DIMITROFF. 1971. Toxic effects of cadmium: a review. Environ. Res. 4: 71–85.

GUNN, S. A., T. C. GOULD, AND W. A. D. ANDERSON. 1963. The selective injurious response of testicular and epididymal blood vessels to cadmium and its prevention by zinc. Amer. J. Pathol. 42: 685–702.

JACKIM, E., J. M. Hamlin, AND S. Sonis. 1970. Effects of metal poisoning on five liver enzymes in the killifish (*Fundulus heteroclitus*). J. Fish. Res. Bd. Canada 27: 383–390.

KOPP, J. F., AND R. C. KRONER 1970. Trace metals in waters of the United States. Fed. Water Pollut. Contr. Admin., U.S. Dep. Interior, Cincinnati, Ohio. 212 p.

LAGERWERFF, J. V., AND A. W. SPECHT. 1970. Contamination of roadside soil and vegetation with cadmium, nickel, lead and zinc. Environ. Sci. Technol. 4: 583–586.

LUCAS, H. F., Jr., D. N. EDGINGTON, AND P. J. COLBY. 1970. Concentrations of trace elements in Great Lakes fishes. J. Fish. Res. Bd. Canada 27: 677–684.

LUEDKE, E. M., C. T. WRUCKE, AND J. A. GRAHAM. 1959. Mineral occurrences of New York State with selected references to each locality. Geol. Surv. Bull. 1072-F, U.S. Govt. Printing Office, Washington, D.C. p. 385–444.

MALIN, M. H., Jr. 1971. Cadmium: potential hazard but no disaster likely. Environ. Sci. Technol. 5: 754–755.

MURPHY, E. W., B. K. WATT, AND L. PAGE. 1970. Regional variations in vitamin and trace element content of Type A school lunches, p. 194–205. *In* D. D. Hemphill [ed.] Trace substances in environmental health. Vol. IV. Proc. Univ. Missouri 4th Annu. Conf. Trace Subst. Environ. Health, Columbia, Mo.

MURTHY, G. K., U. RHEA, AND J. T. PEELER. 1971. Levels of antimony, cadmium, chromium, cobalt, manganese, and zinc in institutional total diets. Environ. Sci. Technol. 5: 436–442.

PERLMUTTER, N. M., M. LIEBER, AND H. L. FRAUENTHAL. 1963. Movement of waterborne cadmium and hexavalent chromium wastes in South Farmingdale, Nassau County, Long Island, New York. U.S. Geol. Surv. Prof. Pap. 475-C, C179–C184.

PISCATOR, M. 1962. Proteinuria in chronic cadmium poisoning. Arch. Environ. Health 4: 607–621.

PRINGLE, B. H., D. E. HISSONG, E. L. KATZ, AND S. T. MILAWKA. 1968. Trace metal accumulation by estuarine mollusks. Proc. Amer. Soc. Civil Eng. Sanit. Eng. Div. 6: 455–475.

SCHROEDER, H. A. 1964. Cadmium hypertension in rats. Amer. J. Physiol. 207: 62–66.

1965. Cadmium as a factor in hypertension. J. Chronic Dis. 18: 647–656.

SCHROEDER, H. A., J. J. BALASSA, AND W. H. VINTON, Jr. 1971. Chromium, cadmium and lead in rats: effects on life span, tumors and tissue levels. J. Nutr. 86: 51–66.

SCHROEDER, H. A., S. S. KROLL, J. W. LITTLE, P. O. LIVINGSTON, AND M. A. G. MYERS. 1966. Hypertension in rats from injection of cadmium. Arch. Environ. Health 13: 788–789.

SCHROEDER, H. A., A. P. NASON, R. E. Prior, J. B. REED, AND W. T. HAESSLER. 1968. Influence of cadmium on renal ischemic hypertension in rats. Amer. J. Physiol. 214: 469–474.

SCHROEDER, H. A., A. P. NASON, I. H. TIPTON, AND J. J. BALASSA. 1967. Essential trace metals in man: zinc. Relation to environmental cadmium. J. Chronic Dis. 20: 179–210.

SCHROEDER, H. A., AND W. H. VINTON, Jr. 1962. Hypertension induced in rats by small doses of cadmium. Amer. J. Physiol. 202: 515–518.

SCHWEIGER, G. 1957. The toxic action of heavy metal salts on fish and organisms on which fish feed. Arch. Fischereiwiss. 8: 54–62.

TRUHAUT, R., AND C. BOUDENE. 1954. Enquiries into the fate of cadmium in the body during poisoning: of special interest to industrial medicine. Arch. Hig. Rada. 5: 19–32.

VIGLIANI, E. C. 1969. The biopathology of cadmium. Amer. Ind. Hyg. Ass. J. 30: 329–340.

Water Quality Criteria. 1968. Report of the National Technical Advisory Committee to the Secretary of the Interior. U.S. Govt. Printing Office, Washington, D.C. 234 p.

# Taxonomy, Prevalence, and Specificity of *Cryptobia dahli* (Möbius) (Mastigophora: Bodonidae) in Lumpfish, *Cyclopterus lumpus*[1]

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KHAN, R. A., AND E. R. NOBLE. 1972. Taxonomy, prevalence, and specificity of *Cryptobia dahli* (Möbius) (Mastigophora: Bodonidae) in lumpfish, *Cyclopterus lumpus*. J. Fish. Res. Bd. Canada 29: 1291–1294.

The protozoan parasite *Cryptobia dahli* (Mastigophora: Bodonidae) is reported from the stomach of lumpfish, *Cyclopterus lumpus*, captured off coastal Newfoundland. The parasite was observed in 104 adult specimens but not in 407 viable eggs, 246 young (< 10 mm) that were reared in the laboratory, or in 57 immature lumpfish (25–130 mm). Experiments on host susceptibility indicate, however, that young lumpfish can become infected with *C. dahli* but not *Myoxocephalus octodecemspinosus*, *Mallotus villosus*, *Pseudopleuronectes americanus*, or *Tautogolabrus adspersus*. It is likely that infections in lumpfish are acquired naturally following ingestion of regurgitated flagellates.

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Les auteurs signalent la présence d'un protozoaire parasite, *Cryptobia dahli* (Mastigophora: Bodonidae), dans l'estomac de grosses poules de mer, *Cyclopterus lumpus*, capturées au large des côtes de Terre-Neuve. Le parasite était présent chez 104 individus adultes, mais absent de 407 oeufs viables, 246 jeunes (< 10 mm) élevés au laboratoire et 57 poissons immatures (25–130 mm). Des essais démontrent cependant que les jeunes poules de mer sont susceptibles à l'infestation par *C. dahli*, alors que *Myoxocephalus octodecemspinosus*, *Mallotus villosus*, *Pseudopleuronectes americanus* et *Tautogolabrus adspersus* ne le sont pas. La grosse poule de mer acquiert probablement le parasite naturellement à la suite de l'ingestion de flagellés régurgités.

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SPECIES of the genus *Cryptobia* in marine fish are parasitic haemoflagellates although a few have become established in the gut of their hosts (Noble 1968). A species was described by Möbius (1888) from the stomach of lumpfish, *Cyclopterus lumpus*, as *Diplomastix dahlii*. Leger (1905) and Keysselitz (1906) mentioned and illustrated, from the stomach of the same host, a parasite which they named *Trypanoplasma intestinalis* and *T. ventriculi*, respectively. Alexeieff (1912) subsequently changed the name to *Cryptobia dahli* (Möbius). Roskin (1923) found this parasite in lumpfish of the North Sea. Bazikolova (1932), apparently unaware of previous reports, described the flagellate as a new genus and new species, *Cryptobiella cyclopterina*, since an undulating membrane was not observed in the specimens examined. Subsequently, Polyanskii (1955) also found this parasite in *C. lumpus*, noting that in a stained specimen the undulating membrane was not readily discernible in consequence of detachment of the posterior flagellum from the body. He considered *Cryptobiella cyclopterina* synonymous with *Cryptobia dahli*.

Lumpfish are common in inshore waters of coastal Newfoundland where they migrate in spring to spawn (Leim and Scott 1966). Examination of a female specimen in June, 1970 revealed an estimated infection of $1.6 \times 10^7$ cryptobiids/cc in its stomach fluid. Parasites were also observed in other parts of

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the alimentary tract but in decreasing numbers. This observation prompted us to investigate the taxonomy, prevalence, and host range of the species of *Cryptobia* in lumpfish.

## Materials and Methods

Adult and juvenile lumpfish were collected in coastal areas adjacent to St. John's, Nfld. Drops of stomach fluid from fish were examined microscopically (approximately 10 min at 400 X) for the presence of *C. dahli* shortly after arrival at the laboratory. Emphasis was placed on the stomach fluid since numbers of parasites in it were considerably greater than in other parts of the digestive tract or in the urinary bladder. Fluid from the stomach of live fish was removed by means of a pipette with a rubber bulb at one end. Infections were estimated with a Levy counting chamber (expressed as the number of parasites/cc of stomach fluid). For permanent preparations, specimens were fixed in Bouin's fluid or iodine vapor and stained with iron-hematoxylin.

Lumpfish egg-masses and juvenile fish were kept in aquaria (50 × 30 × 25 cm) through which there was a constant flow of sea water (flow-rate, 2 liters/min). Fish intended as experimental hosts were held at 9–11 C in tanks (2.0 × 0.8 × 0.8 m) in which sea water flowed (flow-rate, 9 liters/min) and were examined twice weekly over a 4-week period to ensure that they were not infected with species of *Cryptobia* in either their stomachs or blood. In experiments to determine host susceptibility, fluid containing flagellates was fed to recipients by a graduated pipette with a rubber bulb at one end. The parasites were also inoculated intraperitoneally into a number of fish to ascertain whether infections would develop in the blood system of the recipients.

## Results

### Morphology and Taxonomy

The flagellate is elongate, tapered towards the posterior end and rounded at its anterior end. The average body length is 16.4 μ. The nucleus is almost centrally located in the granular cytoplasm. Anterior to the nucleus lies a kinetoplast that is rod-shaped or rounded (Fig. 1) to bilobed (Fig. 2). Two kinetoplasts are sometimes seen (Fig. 3), possibly indicating an early division stage. The two flagella have a common point of origin, the kinetosome, which is anteriorly juxtaposed to the kinetoplast.

An undulating membrane is readily discernible in living specimens from the stomach of infected lumpfish collected in Newfoundland coastal waters, as illustrated in stained preparations in Fig. 2 and 3. Following death, especially after air-drying, it folds over on the body and is not easily seen after staining. Living *C. dahli* from the stomach of *Cyclopterus lumpus* taken from the north coast of Scotland were studied with a phase-contrast microscope at the Fisheries Marine Laboratory at Aberdeen in June, 1971. A typical undulating membrane was not observed, but a membrane was visible as a very narrow ribbon (Fig. 4) whose undulations appeared weak. There are several possible explanations for the different reports in the literature on the presence or absence of an undulating membrane, among them the following: the membrane may be "invisible" because of the fixation and staining methods used; it may be more pronounced in some strains (subspecies) than in others, it may vary with the varying specific chemical environment of the host's digestive tract, or it may reflect differences in the age of the parasite population. Cultivation experiments, using a variety of media and changing physical conditions, might help to determine answers to such speculation.

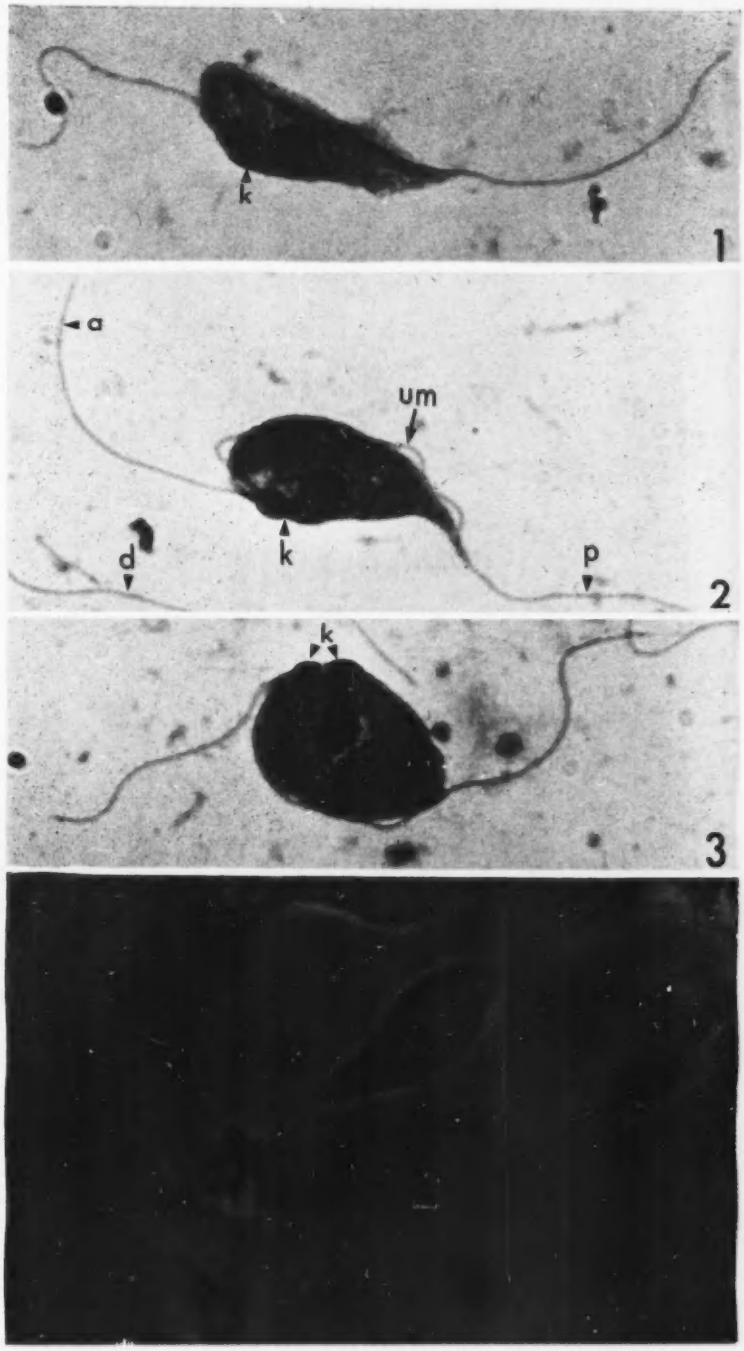
Comparisons of the dimensions of *Cryptobia* in lumpfish from the Barents Sea and from coastal Newfoundland suggest that the differences do not warrant a taxonomic separation (Table 1). We, therefore, consider the flagellate in the digestive tract of lumpfish from coastal Newfoundland to be *C. dahli* (Möbius) Alexeieff. We agree with Alexeieff (1912) that Leger's *Trypanoplasma intestinalis* and Keysselitz's *T. ventriculi* are synonyms of *C. dahli*.

### Prevalence

The survey was initially directed to ascertain the prevalence of *Cryptobia* in adult lumpfish, since these are more readily obtainable than the young. All of 104 adult fish (200–500 mm) examined to date harbored infections of *C. dahli*. There appeared to be no difference in the infection level between the two sexes. Infections in the stomach of fish varied from an estimated $8 \times 10^5$ to $5.3 \times 10^8$ organisms/cc of fluid and are probably the highest recorded for any flagellate in marine fish. Flagellates also occurred in the rest of the alimentary tract posterior to the stomach but in decreasing numbers; they were not

Table 1. Dimensions (μ) of species of *Cryptobia* in lumpfish from the Barents Sea and from coastal Newfoundland (range in parenthesis).

| Characteristic | Species of *Cryptobia* | |
|---|---|---|
| | Barents Sea | Coastal Nfld. |
| Body length | 10–20 | 16.4(13–19) |
| Body width | 4.6 | 4.4 (3–6) |
| Length of nucleus | 2–2.5 | 2.4 (2–3) |
| Kinetoplast (parabasal body) | 3–7 | 4.7 (3–6) |
| Anterior flagellum | 15–35 | 24.7(17–33) |
| Posterior flagellum | 5–15 | 20.5(15–22) |




FIGURES 1–4.

FIG. 1. *C. dahli* with oval-rod-shaped kinetoplast. × 2200; FIG. 2. Bilobed kinetoplast. × 2200; FIG. 3. Divided kinetoplast: a = anterior flagellum, d = detached flagellum from another *C. dahli*, k = kinetoplast, p = posterior flagellum, um = undulating membrane. × 2200; FIG. 4. Phase contrast photomicrograph of *C. dahli* with very thin undulating membrane. × 2000.

found in the blood, or in any internal organ except the urinary bladder.

The studies were next directed towards the immature stages of the host to determine the age at which infection was acquired. Lumpfish egg-masses close to shore were collected by scuba divers. Six such egg masses were held in sea water at 11–14 C. Eggs from the "eyed" stage to young 10 weeks old (<10 mm) were dissected daily to detect the presence of *Cryptobia*. A total of 407 viable eggs from the "eyed" stage, and 246 young up to 10 weeks old (<10 mm) were examined for *C. dahli* but none was found. Older fish were difficult to collect because of their clandestine habits; none of 57 immature fish (25–130 mm in length) harbored the parasite.

### Host Range

Experiments were conducted to ascertain whether *Cryptobia* would develop in marine fish (previously determined to be uninfected) other than lumpfish. Approximately 0.5 cc of stomach fluid containing $1.3 \times 10^7$ organisms/cc were fed to five longhorn sculpins, *Myoxocephalus octodecemspinosus*, 120–220 mm; 10 adult capelin, *Mallotus villosus*, 100–160 mm; five winter flounder, *Pseudopleuronectes americanus*, 120–200 mm; three cunners, *Tautogolabrus adspersus*, 150–200 mm; and five control lumpfish, *Cyclopterus lumpus*, 70–110 mm. All fish were examined daily for 2 weeks. Parasites were observed only in five *C. lumpus* during this period and had increased in numbers exceeding the inoculum. The highest infection in one of the fish was estimated at $1 \times 10^7$ organisms/cc of stomach fluid. Infections in the remaining fish decreased and parasites were not seen from days 4–14. The results suggest that *C. dahli* has a host range that does not include the four genera of marine fish listed above.

Since species of *Cryptobia* have been reported from the blood of fish e.g., *C. bullocki* in winter flounder (Strout 1965), it seemed feasible to determine whether *C. dahli* would become established in the blood vascular system. Consequently, a number of fish, whose blood was proven previously to be uninfected, were inoculated intraperitoneally with 0.5 cc of stomach fluid containing an estimated $2 \times 10^7$ organisms/cc. Recipients were: four winter flounder 180–250 mm; two longhorn sculpins 120 and 160 mm; four cunners 110–150 mm; two cod, *Gadus morhua*, 160 and 180 mm; and two lumpfish 90 and 110 mm. Drops of blood, obtained from the gills of the recipients held at 11–14 C, were examined every other day for 28 days. Parasites were not seen during this period in any of these fish. It would appear from these results that *C. dahli* is limited to the digestive tract and urinary bladder.

## Discussion

Occurrence of *Cryptobia dahli* in lumpfish of the Barents Sea, North Sea, and coastal Newfoundland indicates a wide zoogeographical distribution closely related to the distribution of its host. Finding flagellates only in adult lumpfish does not necessarily imply that infections occur with adulthood. Our experiment on host range, reported above, demonstrated that young fish can become infected with the flagellates. Young lumpfish generally remain inshore, but as maturity approaches they tend to move offshore in winter. Possibly, infections are acquired during these migrations. Noble (1968) has suggested that transmission of *C. stilbia* and *C. coryphanoideana* is probably direct via sea water. We have observed *C. dahli* in a regurgitated mass in a tank with a continuous flow of sea water at 11–14 C, and flagellates, although fewer, were still alive for up to 2 days (unpublished data). It is also possible that lumpfish acquire infections through ingestion of intermediate hosts. This view is supported by the observations that parasites were not seen in 57 immature fish, which were collected in areas frequented by adult lumpfish. However, such a hypothesis awaits demonstration of *C. dahli* in potential carriers such as pelagic crustacea, annelids, jellyfish, and small fish which serve as food for lumpfish (Leim and Scott 1966). On the basis of available information, the more likely mode of infection is via direct transmission. However, much more information is needed to unravel the interrelations of this marine host-parasite complex.

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Alexeieff, A. 1912. Quelques remarques à propos de la specificité parasitaire. Sur le véritable nom de *Cryptobia* (= *Trypanoplasma*) *intestinalis* et sur celui du trypanosome pathogène des mammifères; quelques autres questions de synonymie chez les protozoaires. Zool. Anz. 41: 17–37.

Bazikolova, A. Y. 1932. Materialy po parazitologii Murmanskikh ryb. (Study specimens of fish parasitology), p. 136–153. *In* Sb. Nauch-Promysl. Rab. Murm. Snabtekhizdat. (In Russian)

KEYSSELITZ, G. 1906. Generations und Wirtswechsel von *Trypanoplasma borreli* Laveran et Mesnil. Arch. Protistenk. 7: 1–74.

LEGER, L. 1905. Sur la présence d'un *Trypanoplasma* intestinal chez les poissons. C. R. Soc. Biol. Paris 58: 511–13.

LEIM, A. H., AND W. B. SCOTT. 1966. Fishes of the Atlantic coast of Canada. Fish. Res. Board Can. Bull. 155: 485 p.

MÖBIUS, K. A. 1888. Brüchstucke einer Infusorienfauna der Kieler Bucht. Arch. Naturgesch. 54: 81–116.

NOBLE, E. R. 1968. The flagellate *Cryptobia* in two species of deepsea fishes from the Eastern Pacific. J. Parasitol. 54: 720–4.

POLYANSKII, Y. I. 1955. Parasites of the fish of the Barents Sea. U.S. Dep. Inter. and Nat. Sci. Found., Washington, D.C. 158 p. (orig. publ. in 1955, Izd. Stvo Akad. Nauk SSSR)

ROSKIN, G. 1923. On the structure of *Trypanoplasma dahlii* (Möebius) Arkh. Russ. Protistenk. Obshch. 2: 230–240. (In Russian with German summary)

STROUT, R. G. 1965. A new haemoflagellate (genus (*Cryptobia*) from marine fishes of northern New England. J. Parasitol. 51: 654–659.

# Acute Toxicity of Yellow Phosphorus to Atlantic Cod (*Gadus morhua*) and Atlantic Salmon (*Salmo salar*) Smolts

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FLETCHER, G. L., AND R. J. HOYLE. 1972. Acute toxicity of yellow phosphorus to Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*) smolts. J. Fish. Res. Bd. Canada 29: 1295–1301.

The acute toxicity of yellow phosphorus ($P_4$) to Atlantic cod (*Gadus morhua*) and Atlantic salmon smolts (*Salmo salar*) was investigated using two experimental procedures: (a) continuous exposure and (b) single brief exposure.

In continuous-exposure experiments, $P_4$ was lethal to cod and seawater-maintained salmon at concentrations as low as 1.89 and 0.79 μg/liter, respectively. Salmon that were exposed to $P_4$ concentrations of 40 μg/liter or less developed a distinct external red color and showed signs of extensive hemolysis. At death, salmon that had been exposed to $P_4$ concentrations of 90 μg/liter and lower showed a progressive decline in hematocrits. In contrast, cod were never observed to show any evidence of external redness, hemolysis, or reduced hematocrits.

In the brief-exposure experiments, cod and salmon were subjected (20 min to 3.5 hr) to $P_4$ concentrations ranging from 245 to 4030 μg/liter. These brief exposures resulted in mortalities to both species that were delayed for as long as 2 weeks. Salmon turned "red" 16–24 hr following exposure, and at death showed evidence of hemolysis and reduced hematocrits. None of the cod exhibited any of these symptoms.

FLETCHER, G. L., AND R. J. HOYLE. 1972. Acute toxicity of yellow phosphorus to Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*) smolts. J. Fish. Res. Bd. Canada 29: 1295–1301.

Nous avons poursuivi des recherches sur la toxicité aiguë du phosphore jaune ($P_4$) à l'endroit de la morue (*Gadus morhua*) et des smolts de saumon (*Salmo salar*) suivant deux techniques opératoires: (a) exposition continue, (b) exposition brève unique.

Dans les essais à exposition continue, le $P_4$ cause la mort des morues et des saumons, ces derniers maintenus en eau salée, à des concentrations aussi faibles que 1.89 et 0.79 μg/litre respectivement. Les saumons exposés à des teneurs en $P_4$ de 40 μg/litre ou moins développent une coloration rouge distincte sur la surface du corps et montrent des symptômes de forte hémolyse. A la mort, les saumons exposés à des teneurs en $P_4$ de 90 μg/litre ou moins montrent un déclin progressif des hématocrites. En revanche, la morue ne présente aucun signe de rougeur externe, d'hémolyse ou de diminution d'hématocrites.

Dans les essais à exposition brève, les morues et les saumons furent soumis (de 20 min à 3.5 h) à des teneurs en $P_4$ allant de 245 à 4030 μg/litre. Ces courtes expositions sont mortelles pour les deux espèces, après un délai pouvant aller jusqu'à deux semaines. Les saumons "rougissent" 16 à 24 h après exposition et, à la mort, montrent des signes d'hémolyse et des diminutions d'hématocrites. Ces symptômes sont absents des morues exposées.

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THE yellow phosphorus ($P_4$) pollution of Long Harbour, Nfld, (Idler 1969; Jangaard 1970) prompted a number of investigations into the acute toxicity of this element to several species of teleost fish, principally seawater-maintained brook trout (*Salvelinus fontinalis*) (Fletcher et al. 1970) and herring (*Clupea harengus*) (Zitko et al. 1970). The results of these experiments showed no evidence of an incipient lethal level and demonstrated that $P_4$ was lethal at concentrations as low as 0.5 and 2.5 μg/liter for trout and herring, respectively. These data indicated that this element was 10–100 times more toxic to teleosts than had been previously established (Isom 1960). However, due to mortalities

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of controls, the results from the trout experiments must be interpreted with some caution (Fletcher et al. 1970).

This paper describes experiments on the acute toxicity of $P_4$ to Atlantic salmon (*Salmo salar*), a species more readily adaptable to sea water than brook trout, and Atlantic cod (*Gadus morhua*), one of the principal species of fish killed during the Long Harbour pollution crisis.

## Materials and Methods

Two-year-old Atlantic salmon smolts weighing between 30 and 60 g were obtained from Cobequid Fish Hatchery, N.S., and held in recirculating fresh water (10–12 C) for at least 2 weeks prior to use. A group of them were then gradually adapted to sea water (33‰) by raising the salinity approximately 2‰ daily. These fish were then maintained in sea water for 3 or more weeks prior to experimentation.

All salmon stocks were held under continuous light and fed an ad libitum supply of Purina trout chow daily.

Atlantic cod weighing between 300 and 3000 g were caught by hook and line outside of Halifax Harbor, N.S. At the laboratory they were held in flowing sea water (salinity, 33‰; temperature, 9–11 C) and fed a daily ration of herring for at least 3 weeks prior to use.

The toxicity of $P_4$ to these species of fish was investigated using two experimental procedures. In one the fish were continuously exposed to the element; in the other, fish were subjected to a single brief exposure.

The test tanks used for salmon in the continuous-exposure experiments consisted of horizontal plexiglass cylinders (140 × 15 cm in diameter) (Fletcher et al. MS 1971) that contained approximately 15 liters of water flowing at 1 liter/min (temperature, 10–13 C). All fish were allowed at least 24 hr to adapt to the tank before being subjected to $P_4$. Nineteen experiments were conducted using $P_4$ concentrations ranging from 0.79 to 1900 µg/liter.

Cod were continuously exposed to $P_4$ in 90 × 90 cm fiberglass tanks containing 250 liters of water (9–10 C) flowing at 1 liter/min. The cod were placed in the tanks approximately 2 hr prior to the addition of $P_4$. Sixteen experiments were carried out using $P_4$ concentrations ranging from 1.89 to 5780 µg/liter.

All brief-exposure experiments were carried out in 250-liter tanks. For exposures of an hour or less in duration, $P_4$ suspensions (100–200 ml) were added directly to the tank containing the fish. At the end of the exposure period, the fish were removed from the tanks, rinsed thoroughly in clean water, and transferred to another tank containing clean water flowing at approximately 8–10 liter/min.

For brief-exposure experiments longer than 1 hour, a procedure similar to the continuous-exposure experiments had to be employed. Suspensions of $P_4$ were pumped (3–4 ml/min) into a tank containing fish. When the $P_4$ concentration in the water became constant, the fish in the tank were discarded and replaced with unexposed fish of the same size. This procedure ensured that the experimental fish would be exposed to a constant concentration of $P_4$ for the duration of the exposure. At the end of the exposure period the fish were removed, rinsed, and transferred to clean water.

Five fish were used in each continuous-exposure and brief-exposure experiment. Each fish, when dead, was removed from the tank, weighed, and a hematocrit was measured from a blood sample taken from the caudal artery. LT50s (lethal time for 50% of the individuals) and standard error were determined by probit analysis (Finney 1952) using the IBM 360 computer at Dalhousie University, Halifax, N.S.

None of the fish were fed during the experiment or in the 24 hr preceding it.

Dissolved oxygen remained above 90% of its air saturation value during the course of all experiments.

$P_4$ was suspended in distilled water (60–70 C) with the use of a sonicator. The resulting stock suspension was then either added directly to the bioassay tank or pumped into it (0.5–10 ml/min) at the water inlet. In a number of experiments on salmon, $P_4$ was made up in an ethanolic solution (2 mg/ml) and pumped into the bioassay tank at 0.3–10 ml/hr. Water samples were removed from the tanks at 0.5–2-hr intervals, depending on the length of the experiment, and immediately extracted with 50 ml of benzene. The concentration of $P_4$ was determined using the gas–liquid chromatographic method of Addison and Ackman (1970).

These experiments were conducted during the months of September–December 1969.

## Results

### Continuous-Exposure Experiments

*Salmon* — The results obtained from experiments using ethanolic solutions of $P_4$ were similar to those obtained using aqueous suspensions of $P_4$. Therefore, all of the results from the lethal experiments on seawater-maintained salmon were combined. No evidence of an incipient lethal level was observed, since the lowest concentration of $P_4$ tested (0.79 µg/liter) gave an LT50 of 195 hr. The regression relating LT50 to $P_4$ concentration was $Y = 153X^{-0.555}$. The correlation coefficient was −0.933 ($P < 0.01$).

Three control experiments were performed: two of them controlling for aqueous suspensions of $P_4$ (run for 30 days) and the third (run for 14 days) testing the highest concentration of ethanol used (0.16 ml/liter). No mortalities were observed in any of the control groups.

After a period of time ranging from 20 to 70 hr many of the salmon that were exposed to $P_4$ concentrations of 40 µg/liter or less developed a distinct external red color, particularly around the head and fin regions (Fig. 1).

Only one of the fish in the group exposed to 40.7 µg/liter turned "red" and this fish was the last one to die in that group. All of the fish in

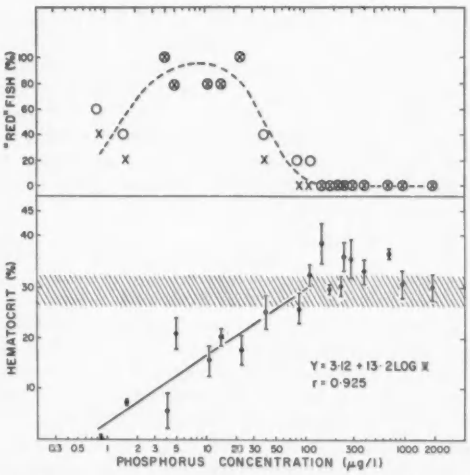


FIG. 1. Continuous-exposure experiments. Relation between hematocrits, hemolysis, and the external redness of seawater-maintained salmon and the concentration of yellow phosphorus to which they were continuously exposed until death. Each group consisted of five salmon. In the upper figure, X represents the percentage of the fish in each group which appeared "red" at death and ○ represents the percentage of the fish in each group where the plasma showed evidence of hemolysis at death. In the lower figure, hematocrits (expressed as mean ±SE for each group) were measured immediately after death. The horizontal shaded area represents the hematocrits of the control fish. The regression was computed from the means using the method of least squares.

the groups exposed to 0.79 and 1.6 µg/liter developed the external red color. However, several of them subsequently lost this coloration prior to death (Fig. 1). For example, all of the salmon exposed to 0.79 µg/liter turned "red" between 40 and 70 hr. The first two fish to die (150 and 200 hr) retained this red color, while the remaining three fish gradually lost the redness before dying. In fact, the gills of the last two fish became almost white prior to death.

At death, the degree and extent of the external red color was correlated with the degree to which the plasma of these fish showed evidence of hemolysis. Extensive hemolysis was evident in all of the fish which appeared "red." In addition, some hemolysis was also evident in several of the normal-appearing fish (Fig. 1). Those fish in the groups exposed to 5, 11, and 15 µg/liter $P_4$ that did not show any evidence of external redness or hemolysis were the first fish in their respective groups to die.

Within the various groups of salmon examined, it was apparent that the hematocrits progressively declined with decreasing phosphorus concentrations below 100 µg/liter (Fig. 1). Furthermore, within a group, those fish that had been exposed to $P_4$ for the longest periods of time had the lowest hematocrits.

The toxicity of $P_4$ to freshwater-maintained salmon smolts (30–60 g) was determined using three concentrations. The experiments were performed as described for the continuously exposed seawater salmon (temperature 11–14 C). The LT50s obtained were 2.82, 20.9, and 50.2 hr for $P_4$ concentrations 763, 99.0, and 22.8 µg/liter, respectively.

None of the salmon exposed to 763 µg/liter showed any evidence of external redness or hemolysis (hematocrit 44.9 ± 1.93 SE). All of the fish in the group exposed to 99 µg/liter had slight hemolysis but no redness (hematocrit 40.3 ± 1.20%), while those fish exposed to 22.8 µg/liter were "red" and had extensive hemolysis and reduced hematocrits (hematocrit 11.0 ± 6.90)%. These data are comparable to those obtained for the seawater-maintained salmon.

*Cod* — No evidence of an incipient lethal level was observed, the lowest concentration of $P_4$ (1.89 µg/liter) producing an LT50 of 125 hr. The regression relating LT50 to $P_4$ concentration was $Y = 159X^{-0.559}$. The correlation coefficient was 0.971 ($P < 0.01$).

Two groups of control experiments were conducted for a period of 30 days. Only one of the cod in these experiments died.

In contrast to the salmon, none of the cod were observed to develop any external red color, nor was there any evidence of hemolysis. The hematocrits, although variable, did not exhibit any significant trends throughout the entire range of phosphorus concentrations tested. The mean hematocrits of control fish and of fish exposed to the lowest concentration of $P_4$ (1.89 µg/liter were 30.5 ± 3.80 (SE) and 37.3 ± 3.60, respectively.

## BRIEF-EXPOSURE EXPERIMENTS

In a series of experiments with cod and salmon it was established that $P_4$ was lethal even when the period of exposure was relatively brief (Table 1). Furthermore, the lethal times observed for these short exposures were considerably longer than what would be expected if the fish had been continuously exposed. For example, in one experiment where cod were exposed to 4030 µg/liter $P_4$ for 0.5 hr, an LT50 of 29.9 hr was obtained. If the cod had been continuously exposed to this concentration they would have died in 1–2 hr.

An examination of the data obtained from the various groups indicated that the LT50s appeared

TABLE 1. Groups of five fish were exposed to yellow phosphorus for the periods of time indicated and then transferred to another tank containing clean water. LT50s (time to 50% mortality) are expressed as ± SE.

| | $P_4$ (*μg/liter*) | Time of exposure (*hr*) | LT50 (*hr*) |
|---|---|---|---|
| Salmon | 2480 | 1.00 | 5.93±0.269 |
| | 2480 | 0.50 | 47.73±17.80 |
| | 2264 | 0.33 | 329.0±73.0 |
| | 2250 | 0.33 | >1000.0 |
| | 1850 | 0.75 | 11.4±2.46 |
| | 1750 | 1.00 | 6.93±0.269 |
| | 1723 | 1.25 | 6.86±0.170 |
| | 1308 | 0.75 | 40.80±19.7 |
| | 1210 | 1.17 | 6.50±0.948 |
| | 1132 | 0.75 | 8.22±0.132 |
| | 808 | 0.83 | 134.00±53.3 |
| | 245 | 3.50 | 8.77±0.917 |
| Cod | 4030 | 0.50 | 29.9±6.75 |
| | 3600 | 0.50 | 26.9±4.78 |
| | 3220 | 0.50 | 22.6±2.70 |
| | 2400 | 1.00 | 15.8±4.16 |
| | 2152 | 1.00 | 9.87±4.01 |
| | 1800 | 0.50 | 43.7±6.53 |
| | 1360 | 1.00 | 8.84±3.44 |
| | 1173 | 3.50 | 8.41±2.18 |
| | 1170 | 2.25 | 11.40±0.997 |
| | 1170 | 1.50 | 18.00±3.31 |
| | 1152 | 0.50 | 66.8±19.1 |
| | 1120 | 1.00 | 19.1±5.37 |
| | 956 | 0.50 | >1000 |
| | 820 | 0.50 | 370.0±149.0 |
| | 630 | 3.00 | 25.9±4.70 |
| | 504 | 0.50 | >1000 |

to be related more to the product of the $P_4$ concentration and the time of exposure than to either time or concentration alone. The relation between LT50 and phosphorus exposure expressed as the product of time and concentration (μg–hr/liter) was plotted for both salmon and cod in Fig. 2.

Although the data obtained was quite variable it was apparent that the two species were quite similar in their lethal responses to the brief phosphorus exposures. However, some small differences between the species did exist. For example, a comparison of the LT50s obtained from the salmon exposed to $P_4$ brief exposures of 1300–2500 μg–hr/liter (5 groups) with the LT50s obtained from the corresponding eight cod groups revealed that the cod took significantly longer to die ($P < 0.01$).

Most of the salmon that were exposed to $P_4$ brief exposures of less than 1400 μg–hr/liter exhibited some degree of external redness and hemolysis. The hematocrits of these various groups of salmon are plotted in Fig. 3. Within groups, those fish that survived the longest periods of time displayed the greatest degree of external redness and hemolysis and had the lowest hematocrits. The salmon that turned "red" did so some 16–24 hr following the initial exposure. A few of the fish that died prior to 16 hr also showed evidence of hemolysis.

None of the cod exposed to these brief-exposure experiments exhibited any external red color, hemolysis, or reduced hematocrits.

## Discussion

The evidence obtained from the continuous-exposure experiments indicated that $P_4$ was lethal to seawater-maintained salmon and cod at concentrations as low as 0.79 and 1.89 μg/liter, respectively. However, since no indication of a lethal threshold concentration (Sprague 1970a) was observed it is probable that concentrations below these values would also be lethal. These data agree to a considerable extent with results obtained from previous experiments using seawater-maintained brook trout and smelt (Fletcher et al. 1970). In fact, the almost identical nature of the toxicity curves (regressions relating LT50s and $P_4$ concentrations) obtained for the cod, salmon, and brook trout was remarkable. The data obtained for these species was also similar to that reported by Zitko et al. (1970) for herring (*Clupea harengus*).

The predominant features of $P_4$ poisoning in salmon were external redness, hemolysis, and reduced hematocrits. The close temporal relations observed between the appearance of these symptoms indicated the following sequence of events. Directly or indirectly, $P_4$ caused the hemolysis of the salmon erythrocytes. Once the hemoglobin had entered the plasma some of it then left the circulatory system and entered interstitial areas, imparting to the fish a red external appearance that was particularly obvious in regions that were normally only lightly pigmented. When most of the red cells had hemolyzed, the intensity of the external redness and the concentration of hemoglobin in the plasma declined to negligible levels. At this time excessive amounts of bile pigments were observed in the intestines of the salmon, indicating that the liver had been clearing the hemoglobin from the plasma. Although this entire sequence only occurred in salmon exposed to low $P_4$ concentrations (0.79 and 1.89 μg/liter), various steps in the sequence were evident in the salmon exposed to higher concentrations.

Considering the results from the continuous-exposure and brief-exposure experiments, it was apparent that the effects of $P_4$ on the red blood cells were not simply related to the concentration of $P_4$ in the water per se but rather to some combi-

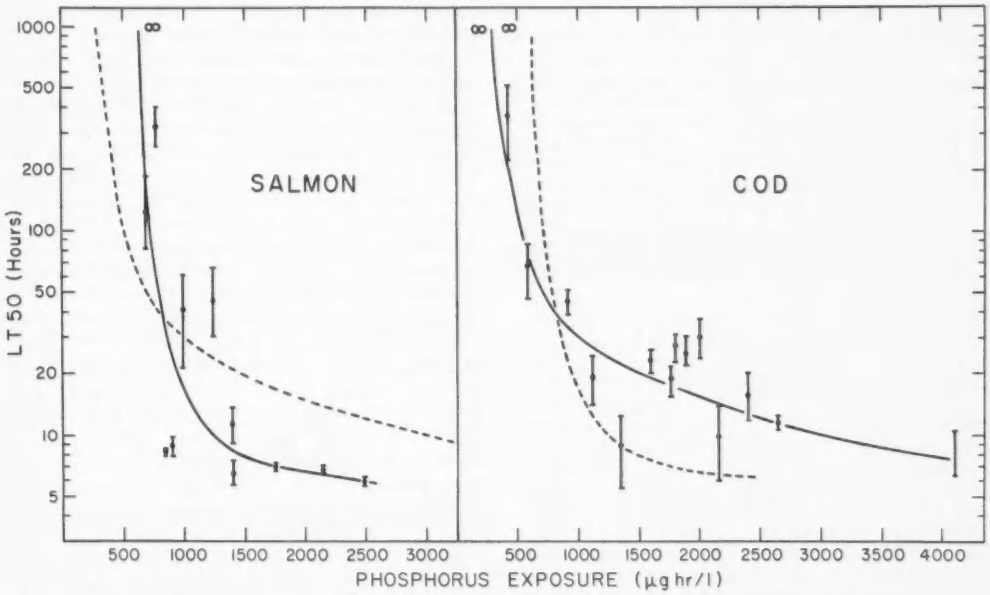


FIG. 2. Brief-exposure experiments. Relation between LT50s of the seawater-maintained salmon and cod and their phosphorus exposure. Groups of five fish were exposed to yellow phosphorus for short periods of time (20 min to 3.5 hr) and then transferred to another tank containing clean water. The phosphorus exposure is expressed as the product of the yellow phosphorus concentration and the time of exposure. LT50s are expressed as mean ±SE. Each ∞ represents a group of fish that did not die during the observation period (1000 hr). The solid line in each graph was drawn freehand through the points to facilitate a comparison of the two species. The broken line in each graph represents the position of the solid line in the contiguous graph.

nation of $P_4$ concentration and time. The role of the latter was particularly evident in the brief-exposure experiments, where it was apparent that a certain period of time was necessary for the effects of phosphorus to manifest themselves.

The various species of fish so far studied differed considerably with respect to the concentration and time of exposure required, for hemolysis to occur. Herring appeared to be the most susceptible for extensive hemolysis was evident after only a few hours (1–3 hr) of exposure (Zitko et al. 1970). In salmon the earliest evidence of hemolysis occurred between 16 and 20 hr, while in trout it was only observed after 50 hr of exposure. Cod did not exhibit any evidence of hemolysis in either the continuous-exposure experiments (150 hr) or the brief-exposure experiment (up to 1000 hr of observation). Preliminary studies with smelt (Fletcher et al. 1970) indicate results similar to those obtained for cod, for no evidence of hemolysis has been observed.

During the Long Harbour pollution crisis, "red" herring were observed dead and dying up to 60 miles from the source of $P_4$ contamination. (Idler 1969; Jangaard 1970; Zitko et al. 1970). Since herring were migrating around Placentia Bay at this time it seemed possible that the dead and dying herring could have passed through the contaminated region and thus been exposed to $P_4$ for a short period of time. This hypothesis was supported by evidence from the brief exposure-experiments with cod and salmon, which demonstrated that short exposures to $P_4$ could result in mortalities that are delayed for at least as long as 2 weeks.

When laboratory results are to be interpreted in terms of field conditions involving migrating fish it is evident that experiments of the brief-exposure type resemble the environmental situation more closely than continuous-exposure experiments. One difficulty, however, is expressing the results in some meaningful and useful form. The suggestion by Zitko et al. (1970) that lethal times could be related to a time–concentration index seemed appropriate; thus, the results from the cod and salmon experiments were plotted in this manner (Fig. 2). Expressed in this form the data

from the various experiments presented a fairly consistent picture, forming a basis on which to predict the outcome of specific environmental conditions and further laboratory experiments.

One of the questions that arose during the course of the brief-exposure experiments was: how do their results relate to those obtained from the continuous-exposure experiments? A comparison of the two sets of data (Fig. 4) indicated that they were reasonably similar. Thus, it would appear that short exposures to relatively high concentrations of $P_4$ were lethal in about the same period of time as were continuous exposures to low concentrations. One further point of interest was that the phosphorus exposure that produced the earliest evidence of hemolysis in the brief-exposure experiments (1390 µg–hr/liter) was similar to that in the continuous-exposure experiments (1800 µg–hr/liter).

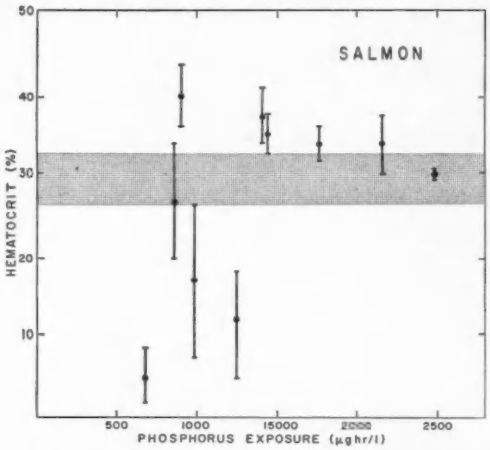


FIG. 3. Brief-exposure experiments. Relation between the hematocrits of the seawater-maintained salmon and phosphorus exposure. Groups of five fish were exposed to yellow phosphorus for brief periods of time (20 min to 3.5 hr) and then transferred to another tank containing clean water. The phosphorus exposure is the product of the yellow phosphorus concentration and the time of exposure. Hematocrits, expressed as mean ±SE for each group, were measured immediately after death. The horizontal shaded area represents the hematocrits of the control fish.

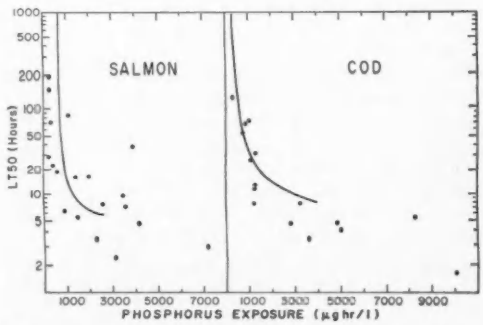


FIG. 4. A comparison of the LT50s obtained from the continuous-exposure and the brief-exposure experiments. Each point represents a group of five fish that were continuously exposed to yellow phosphorus until death. The phosphorus exposure (abscissa) is the product of the yellow phosphorus concentrations and the time taken for the last fish in each group to die. The solid line represents the data obtained for the brief-exposure experiments (as drawn in Fig. 2).

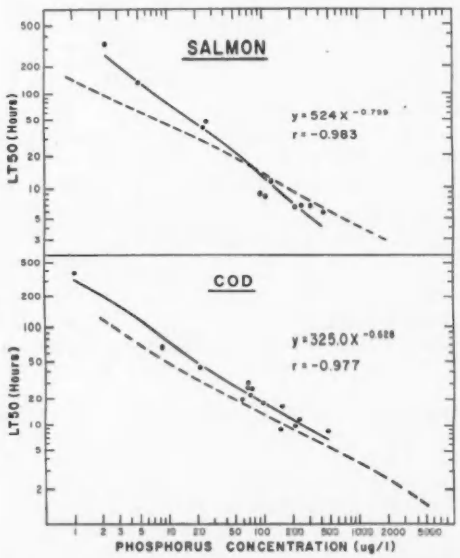


FIG. 5. A comparison of the LT50s obtained from the continuous-exposure experiments and the brief-exposure experiments. Each point represents a brief-exposure experiment. Groups containing five fish were exposed to yellow phosphorus for brief periods of time (20 min to 3.5 hr) and then transferred to another tank containing clean water. The average yellow phosphorus concentration was calculated for these brief-exposure experiments using the expression $\frac{[P_4] \times T}{LT50}$, where $[P_4]$ is the concentration (µgrams per liter) of yellow phosphorus to which the fish were exposed, T is the time (*hr*) the fish were exposed and LT50 the time (*hr*) to 50% mortality. The regression (solid line) was computed by the method of least squares. The broken line represents the regression obtained for the continuous-exposure experiments.

In a recent review, Sprague (1970b) discussed several techniques for handling data from experiments involving fluctuating concentrations of toxicants. Since the present brief-exposure experiments can be considered as one extreme of the fluctuating-concentrations situation, several aspects of the techniques discussed seemed appropriate. For example, experiments by Abram (1967) and Brown et al. (1969) gave evidence that the average of fluctuating concentrations could be used successfully to predict survival times.

An average $P_4$ concentration can be calculated for the brief exposure experiments using the following expression: $\frac{[P_4] \times T}{LT50}$, where $[P_4]$ is the concentration (μgrams per liter) of $P_4$ to which the fish were exposed, T is the time (hours) the fish were exposed, and LT50 the time (hours) to 50% mortality. The relation between the LT50 and the average phosphorus concentration for the brief-exposure experiments was compared with that for the continuous-exposure experiments in Fig. 5. As could be anticipated from Fig. 4, the two regressions do not differ greatly. However, by expressing the data in this manner, the brief-exposure and continuous-exposure experiments could be compared in more detail. Using an analysis of covariance (Snedecor 1956), the slopes of the salmon regressions were found to differ significantly ($F = 5.90$, $P < 0.05$) although their elevations were similar ($F = 1.97$). This indicates that those salmon subjected to relatively low exposures of $P_4$ tended to take longer to die than those continuously exposed. The regression for the cod data showed no difference in their slopes ($F = 0.608$). Their elevations, however, differed significantly ($F = 15.9$, $P < 0.01$), indicating that cod subjected to brief exposures of $P_4$ take longer to die than those exposed continuously.

To date there is no conclusive evidence indicating the cause of death in the fish species studied. Zitko et al. (1970) suggested that in herring, death was due to asphyxiation brought on by massive hemolysis of the red blood cells. Our experiments with salmon, cod, trout, and smelt do not support this view. Although the loss of red blood cells may contribute to the death of some fish species, the fact that cod and smelt have never exhibited any evidence of hemolysis indicates that death was caused by some other means.

A similar conclusion is indicated from the salmon and trout experiments where high concentrations of $P_4$ killed the fish without causing any change in hematocrits.

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ABRAM, F. S. H. 1967. The definition and measurement of fish toxicity thresholds, p. 75–95. *In* Advances in water pollution research. Vol. 1. Proc. 3rd. Int. Conf., Munich, 1966. Pergamon Press Ltd., Oxford, England.

ADDISON, R. F., AND R. G. ACKMAN. 1970. Direct determination of elemental phosphorus by gas-liquid chromatography. J. Chromatogr. 47: 421–426.

BROWN, V. M., D. H. M. JORDAN, AND B. A. TILLER. 1969. The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol, and zinc. J. Fish. Biol. 1: 1–9.

FINNEY, D. J. 1952. Probit Analysis. 2nd ed. Cambridge University Press, London, England, 320 p.

FLETCHER, G. L., R. J. HOYLE, AND D. A. HORNE. 1970. Yellow phosphorus pollution: its toxicity to seawater-maintained brook trout (*Salvelinus fontinalis*) and smelt (*Osmerus mordax*). J. Fish. Res. Bd. Canada 27: 1379–1384.

MS 1971. The relative toxicities of yellow phosphorus production wastes to seawater-maintained fish. Fish. Res. Board Can. Tech. Rep. 255: 14 p.

IDLER, D. R. 1969. Coexistence of a fishery and a major industry in Placentia Bay. Chem. Can. 21: 16–21.

ISOM, B. G. 1960. Toxicity of elemental phosphorus. J. Water Pollut. Contr. Fed. 32: 1312–1316.

JANGAARD, P. M. 1970. The role played by the Fisheries Research Board of Canada in the "red" herring phosphorus pollution crisis in Placentia Bay, Newfoundland. Fish. Res. Board Can. Gen. Ser. Circ. 1: 20 p.

SNEDECOR, G. W. 1956. Statistical methods. 5th ed. Iowa State University Press, Ames, Iowa. 593 p.

SPRAGUE, J. B. 1970a. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

1970b. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4: 3–32.

ZITKO, V., D. E. AIKEN, S. N. TIBBO, K. W. T. BESCH, AND J. M. ANDERSON. 1970. Toxicity of yellow phosphorus to herring (*Clupea harengus*), Atlantic salmon (*Salmo salar*), lobster (*Homarus americanus*), and beach flea (*Gammarus oceanicus*). J. Fish. Res. Bd. Canada 27: 21–29.

# The Preservation of Some Oceanic Animals for Lipid Analysis

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MORRIS, R. J. 1972. The preservation of some oceanic animals for lipid analysis. J. Fish. Res. Bd. Canada 29: 1303–1307.

Preservation experiments were carried out on zooplankton required for lipid analysis. Deep-freezing of the material under nitrogen, after as much surface water as possible has been removed, was found to be a satisfactory method of preservation for periods of up to 9 months. Formalin and methanol methods of preservation resulted in hydrolysis of the animal's lipid and degradation of the polyunsaturated fatty acids.

MORRIS, R. J. 1972. The preservation of some oceanic animals for lipid analysis. J. Fish. Res. Bd. Canada 29: 1303–1307.

L'auteur a effectué sur du zooplancton des essais de préservation nécessaire à l'analyse des lipides. La congélation sous azote du matériel, libéré autant que possible de toute eau de surface, assure une préservation satisfaisante pour des périodes allant jusqu'à 9 mois. La formaline et le méthanol, comme préservatifs, causent l'hydrolyse des lipides de l'animal et la dégradation des acides gras polyinsaturés.

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IN studies of the chemical composition of marine organisms, it is often difficult or impossible to carry out the complete analytical operation on board ship. In such cases it is necessary to preserve the specimens between the time of their collection and their arrival in a shore-based laboratory for examination. For studies of marine lipids, storage in an inert atmosphere at low temperature (–20 C) is generally regarded as satisfactory, but to the best of our knowledge there have been no studies of the effects of such storage on lipid composition. Preservation of material in formaldehyde is not widely used when the material is required for chemical analysis, but it is interesting that Ackman and Eaton (1966) found that although formalin removed some lipid from a preserved sample of euphausiid, the fatty acid composition of this extracted lipid was similar to that of the preserved sample, and hence they concluded that formalin preservation did not alter the fatty acid composition.

In order to study possible changes in chemical composition during storage, the primary requirement is a fairly large quantity of homogeneous material, such as a single species of zooplankton, part of which can be analyzed or extracted immediately on capture, the remainder being preserved and analyzed at intervals. During a recent cruise on R.R.S. *Discovery* in the northeast Atlantic, large swarms of *Meganyctiphanes norvegica* and *Myctophum punctatum* were encountered, and the opportunity was taken to preserve large samples of these by several different methods. This paper presents the results of fatty acid analyses carried out on this material over a period of several months.

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## Materials and Methods

The animals used in this study were caught by net on a biological cruise of R.R.S. *Discovery*, in the northeast Atlantic (September–November 1970). A large sample of *Meganyctiphanes norvegica* was taken at a single net station and separated into lots which were treated as follows: (a) Animals lipids were extracted and stored in chloroform/methanol (2:1) under nitrogen at –30 C; (b) Animals were carefully dried with filter paper to remove as much surface water as possible, and then stored in specially cleaned tubes under an atmosphere of pure nitrogen at –30 C; (c) Animals were preserved in a 7½% solution of formalin[1]; (d) Animals were preserved in a 10% solution of formalin[1]; (e) Animals were preserved in 100% absolute methanol.[2]

At another net station a large number of *Myctophum punctatum* were taken and similar preservation experiments performed, this time 70% methanol being used as a method of preservation instead of 100% methanol, with the animals being fixed in 10% formalin prior to being placed in the methanol.

[1]40% (by weight) solution of formaldehyde (38/10 grade Synthite Ltd U.K.) which had been neutralized by addition of hexamine.

[2]Analar reagent B.D.H. Chemicals Ltd., U.K.

Analyses of the material were carried out at our shore laboratory. Wherever possible, operations were carried out under an atmosphere of oxygen-free nitrogen. Total lipids were extracted by homogenizing the animals with chloroform/methanol (2:1) (Folch et al. 1956). Saponification and determination of non-saponifiable material were carried out by standard methods (American Oil Chemists Society 1965). Thin-layer chromatography of the lipid extracts and preparation, hydrogenation, and gas–liquid chromatographic analyses of the methyl esters of the constituent fatty acids were carried out by methods previously described (Culkin and Morris 1969, 1970).

Samples of the formalin and methanol in which the animals were preserved were also extracted and analyzed by thin-layer chromatography to ascertain whether the preservation solutions had removed any of the major lipid fractions.

## Results

Details on preservation of the animals used in this investigation are given in Table 1, and their fatty acid compositions in Tables 2 and 3.

In the freshly extracted *Meganyctiphanes norvegica*, triglycerides phospholipids, sterols, and diglycerides were found to be the major lipid fractions. Monoglycerides, free fatty acids, hydrocarbons, and sterol esters were present as much smaller fractions. These evaluations were based on visual comparisons of thin-layer chromatographic lipid spots. The lipid content, expressed as percentage wet weight, increased with the period of deep freezing probably due to some dehydration, although there appeared to be a decrease in non-saponifiable content. Thin-layer chromatography of the lipid extracts taken from the deep-frozen material at 2-, 4-, and 9-month intervals, gave similar results to that of the freshly extracted lipids, the monoglycerides being slightly higher. The lipid extracts of the material kept in formalin and methanol, although having a distribution of major fractions similar to that found in the freshly extracted lipids, did contain higher levels of free fatty acids. In addition there had been a considerable loss of lipid into the preservation solutions. Though there did not appear to have been any preferential removal of particular lipid fractions, significant amounts of free fatty acids in the preservation solutions suggested that lipid hydrolysis had taken place. The thin-layer chromatographic analyses of the lipids taken from the samples of *Myctophum punctatum* gave similar results to those of *Meganyctiphanes norvegica*.

Whereas only slight variation was found between the fatty acid analyses of the deep frozen and freshly extracted samples, the formalin- and methanol-preserved samples showed differences in composition well outside the normal limits of experimental error. In the latter samples, the levels of polyunsaturated fatty acids were lower, *Myctophum punctatum* showing a loss of about 50%.

TABLE 1. Preservation experiments on *Meganyctiphanes norvegica* and *Myctophum punctatum* caught in October 1970 in the northeast Atlantic (40°N 20°W).

| Sample | Total lipid (% wet wt) | Non-saponifiable (% total lipid) |
|---|---|---|
| *M. norvegica — net at 510–600 m depth (day)* | | |
| Extracted at sea | – | 2.3 |
| Frozen 2 months | 1.94 | 2.3 |
| Frozen 4 months | 2.04 | 1.2 |
| Frozen 9 months | 2.52 | 1.0 |
| Formalin 7½% 4 months | 1.13 (+0.67 in formalin) | 2.5 |
| Formalin 7½% 9 months | 1.30 (wt. in formalin not determined) | 0.9 |
| Formalin 10% 4 months | 1.64 (+0.64 in formalin) | 2.8 |
| Formalin 10% 9 months | 1.60 (wt. in formalin not determined) | 1.5 |
| Methanol 100% 4 months | 1.20 (+1.10 in methanol) | 1.8 |
| *M. punctatum — net at surface (night)* | | |
| Extracted at sea | – | 1.6 |
| Frozen 4 months | 2.49 | 0.5 |
| Formalin 7½% 4 months | 2.16 (+0.32 in formalin) | 0.5 |
| Methanol 70% 4 months | 1.27 (+0.73 in methanol) | 0.7 |

TABLE 2. Effect of various methods of preservation on the fatty acid composition of *Meganyctiphanes norvegica*.

| Fatty acid | | Double bond position | Extracted at sea | Frozen 2 months | Frozen 4 months | Frozen 9 months | Formalin 7½ 4 months | Formalin 7½ 9 months | Formalin 10 4 months | Formalin 10 9 months | MeOH 100 4 months |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sat | 12:0 | | 0.37 | 0.27 | 0.31 | 0.22 | 0.33 | 0.19 | 0.26 | 0.23 | 0.26 |
| | 13:0 | | 0.17 | 0.16 | 0.14 | 0.13 | 0.16 | 0.10 | 0.13 | 0.08 | 0.15 |
| | 14:0 | | 10.00 | 9.37 | 9.21 | 8.05 | 9.83 | 7.93 | 9.20 | 9.98 | 11.74 |
| | 15:0 | | 2.33 | 2.04 | 2.00 | 2.00 | 2.23 | 2.19 | 2.07 | 2.03 | 2.48 |
| | 16:0 | | 26.47 | 24.62 | 23.71 | 22.32 | 28.08 | 24.73 | 27.48 | 26.52 | 24.46 |
| | 17:0 | | 0.80 | 0.81 | 1.03 | 0.83 | 1.40 | 0.51 | 0.97 | 0.78 | 0.89 |
| | 18:0 | | 0.91 | 1.02 | 1.23 | 2.26 | 1.28 | 2.28 | 0.91 | 2.65 | 1.47 |
| | 19:0 | | 0.79 | 0.79 | 0.67 | 0.91 | 0.84 | 0.76 | 0.87 | 0.82 | 0.82 |
| Total | | | 41.84 | 39.08 | 38.30 | 36.72 | 44.15 | 38.67 | 41.89 | 43.09 | 42.27 |
| Mono | | | | | | | | | | | |
| | 16:1 | 7 | 2.04 | 1.95 | 2.83 | 3.65 | 4.09 | 4.12 | 2.72 | 3.51 | 2.92 |
| | 17:1 | | 0.81 | 0.81 | 0.93 | 0.96 | 0.99 | 1.05 | 0.75 | 1.17 | 0.89 |
| | 18:1 | 9 | 19.50 | 19.64 | 18.88 | 19.01 | 20.24 | 20.41 | 20.54 | 20.28 | 18.91 |
| | 20:1 | 11 | 1.67 | 1.70 | 1.30 | 2.58 | 3.50 | 4.00 | 2.20 | 4.27 | 4.50 |
| | 22:1 | 13 | 0.27 | 0.44 | 0.43 | 0.62 | 0.41 | 1.51 | 0.39 | 1.60 | 0.59 |
| Total | | | 24.29 | 24.54 | 24.37 | 26.82 | 29.23 | 31.09 | 26.60 | 30.83 | 27.81 |
| Poly | | | | | | | | | | | |
| | 18:2 | 9,12 | 0.83 | 1.40 | 1.50 | 1.41 | 0.89 | 1.55 | 1.73 | 1.52 | 0.63 |
| | 18:3 | 6,9,12 | – | – | – | – | – | – | – | – | – |
| | 18:3 | 9,12,15 | 1.00 | 1.05 | 0.96 | 0.90 | 0.80 | 0.72 | 1.01 | 0.80 | 0.99 |
| | 18:4 | 6,9,12,15 | 2.43 | 2.36 | 1.63 | 1.61 | 1.98 | 1.52 | 1.94 | 1.17 | 2.60 |
| | 20:4 | 8,11,14,17 | T | T | T | 0.30 | T | 0.22 | T | 0.23 | T |
| | 20:4 | 5,8,11,14 | 1.25 | 1.80 | 2.06 | 1.60 | 1.65 | 0.80 | 1.94 | 0.90 | 1.65 |
| | 20:5 | 5,8,11,14,17 | 9.16 | 8.93 | 8.72 | 8.87 | 6.24 | 7.24 | 8.20 | 6.71 | 6.34 |
| | 22:4 | 10,13,16,19 | 0.24 | 0.38 | 0.60 | 0.48 | 0.54 | 0.67 | 0.26 | 0.47 | – |
| | 22:4 | 7,10,13,16 | – | – | – | – | – | – | – | – | – |
| | 22:5 | 7,10,13,16,19 | T | T | 0.28 | 0.52 | T | 0.38 | T | 0.31 | T |
| | 22:5 | 4,7,10,13,16 | T | T | T | 0.39 | T | 0.38 | T | 0.31 | T |
| | 22:6 | 4,7,10,13,16,19 | 18.93 | 20.43 | 21.45 | 20.31 | 14.54 | 16.16 | 16.46 | 13.57 | 17.35 |
| Total | | | 33.84 | 36.35 | 37.20 | 36.39 | 26.64 | 29.74 | 31.54 | 25.99 | 29.56 |
| Branched | | | | | | | | | | | |
| | iso14 | | T | T | T | T | T | – | T | T | T |
| | iso16 | | T[a] | T[a] | T[a] | T[a] | T[a] | T[a] | T[a] | T[a] | T[a] |
| | iso18 | | T | T | 0.10 | T | T | T | T | T | T |
| | ante15 | | –[b] | –[b] | –[b] | –[b] | –[b] | –[b] | –[b] | –[b] | –[b] |
| TMTD | | | T[b] | T[b] | T[b] | T[b] | T[b] | T[b] | T[b] | T[b] | T[b] |
| Pristanic | | | –[a] | –[a] | –[a] | –[a] | –[a] | –[a] | –[a] | –[a] | –[a] |

[a]Limit of detectability $< 0.1\%$.
[b]Not separated; TMTD: 4, 8, 12–Trimethyl tridecanoic acid.

## Discussion

It has been found that marine lipids, kept in a solution of chloroform/methanol under pure nitrogen at −30 C and for periods of less than 2 months, undergo a minimum of chemical degradation (R. J. Morris unpublished data). Hence the results for the freshly extracted lipids are considered to be representative of the true composition of the animal's lipid. Comparing the results of the various methods of preservation with the freshly extracted material, deep freezing certianly appears to be the most satisfactory means of storage. Little or no change in chemical composition for up to 9 months deep-frozen storage was indicated by the analytical results. Several changes, however, are apparent in the formalin- and methanol-preserved material. Firstly, up to a third of the animal's total lipids were lost to the preserving solution, although this did appear to be a general lipid loss, and so would probably not affect

TABLE 3. Effect of various methods of preservation on the fatty acid composition of *Myctophum punctatum*.

| Fatty acid | | Double bond position | Extracted at sea | Frozen 4 months | Formalin 7½ 4 months | Methanol 70 4 months |
|---|---|---|---|---|---|---|
| Sat | 12:0 | | 0.10 | T | 0.12 | 0.13 |
| | 13:0 | | T | T | T | T |
| | 14:0 | | 4.05 | 3.16 | 4.32 | 5.07 |
| | 15:0 | | 1.31 | 0.78 | 1.20 | 1.18 |
| | 16:0 | | 24.99 | 27.08 | 32.84 | 31.99 |
| | 17:0 | | 1.67 | 1.93 | 2.52 | 2.45 |
| | 18:0 | | 4.05 | 3.53 | 4.80 | 4.37 |
| | 19:0 | | 0.60 | 0.80 | 0.70 | 0.80 |
| Total | | | 36.77 | 37.35 | 46.50 | 47.99 |
| Mono | | | | | | |
| | 16:1 | 7 | 5.00 | 5.02 | 7.02 | 6.86 |
| | 17:1 | | 0.95 | 1.04 | 1.12 | 1.05 |
| | 18:1 | 9 | 19.75 | 22.92 | 28.00 | 25.30 |
| | 20:1 | 11 | 1.10 | 1.40 | 1.50 | 2.50 |
| | 22:1 | 13 | T | T | T | 0.24 |
| Total | | | 26.80 | 30.38 | 37.82 | 35.95 |
| Poly | 18:2 | 9,12 | 0.47 | 0.50 | 0.26 | 0.25 |
| | 18:3 | 6,9,12 | – | – | – | – |
| | 18:3 | 9,12,15 | 0.57 | 0.72 | 0.54 | 0.69 |
| | 18:4 | 6,9,12,15 | 1.07 | 0.78 | 0.36 | 0.61 |
| | 20:4 | 8,11,14,17 | 0.71 | 0.56 | 0.32 | 0.50 |
| | 20:4 | 5,8,11,14 | 2.02 | 1.67 | 1.60 | 1.31 |
| | 20:5 | 5,8,11,14,17 | 6.07 | 6.58 | 3.12 | 4.68 |
| | 22:4 | 10,13,16,19 | 0.28 | 0.32 | T | T |
| | 22:4 | 7,10,13,16 | 0.24 | T | – | T |
| | 22:5 | 7,10,13,16,19 | 0.59 | 0.56 | T | T |
| | 22:5 | 4,7,10,13,16 | 0.20 | 0.20 | T | T |
| | 22:6 | 4,7,10,13,16,19 | 22.61 | 20.31 | 9.60 | 9.96 |
| Total | | | 34.83 | 32.20 | 15.80 | 18.00 |
| Branched | | | | | | |
| | iso14 | | T | T | – | – |
| | iso16 | | T[a] | T[a] | T[a] | T[a] |
| | iso18 | | T | T | T | T |
| | ante15 | | –[b] | –[b] | –[b] | –[b] |
| TMTD | | | T[b] | T[b] | T[b] | T[b] |
| Pristanic | | | –[a] | –[a] | –[a] | –[a] |

[a]Limit of detectability $< 0.1\%$.
[b]Not separated; TMTD: 4, 8, 12–Trimethyl tridecanoic acid.

the relative fatty acid composition. Secondly, there appeared to have been degradation of the polyunsaturated fatty acids in the lipids, especially in *Myctophum punctatum*. Finally, the increase in the free fatty acid fraction indicated that hydrolysis of the lipids had taken place.

It is suggested that zooplankton may be stored for periods of up to 9 months by the method of deep freezing used in these experiments, with no change to the chemical composition of the animal's lipids. Formalin or methanol methods of preservation were not found to be satisfactory, as lipid hydrolysis and chemical degradation occurred during storage.

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ACKMAN, R. G., AND C. A. EATON. 1966. Lipids of the fin whale (*Balaenoptera physalus*) from North Atlantic waters. III. Occurrence of eicosenoic and docosenoic acids in the zooplankter *Meganyctiphanes norvegica* (M.Sars) and their effect on whale oil composition. Can. J. Biochem. 44: 1561–1566.

AMERICAN OIL CHEMISTS' SOCIETY. 1965. Official and tentative methods. American Oil Chemists' Society, Chicago, Ill.

CULKIN, F., AND R. J. MORRIS. 1969. The fatty acids of some marine crustaceans. Deep-Sea Res. 16: 109–116.

1970. The fatty acids of some cephalopods. Deep-Sea Res. 17: 171–174.

FOLCH, J., M. LEES, AND G. H. S. STANLEY. 1956. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509.

# Toxicity of Hydrogen Sulfide to Goldfish (*Carassius auratus*) as Influenced by Temperature, Oxygen, and Bioassay Techniques[1]

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ADELMAN, I. R., AND L. L. SMITH, JR. 1972. Toxicity of hydrogen sulfide to goldfish (*Carassius auratus*) as influenced by temperature, oxygen, and bioassay techniques. J. Fish. Res. Bd. Canada 29: 1309–1317.

Bioassays were conducted to test the effect of temperature and oxygen on $H_2S$ toxicity to goldfish (*Carassius auratus* L.) and to investigate some factors that influence bioassay results. Relation of $H_2S$ toxicity to temperature is negatively logarithmic over the range of 6.5–25 C. The mean 96-hr TL50 at 6 C was 530 μg/liter and at 25 C was 4 μg/liter. At temperatures of 14, 20, and 26 C, most acute mortality from $H_2S$ ended by 11 days and the 11-day TL50's at these temperatures were significantly different. In bioassays with and without prior oxygen acclimation, decreasing oxygen concentrations increased $H_2S$ toxicity. In the former, mean TL50's were 62 and 48 μg/liter $H_2S$ at oxygen concentrations of 6 and 1.5 mg/liter, respectively, and in the latter, 71 and 53 μg/liter $H_2S$ at the same oxygen concentrations. Variability in bioassay results was not affected by test temperatures of 14, 20, and 26 C, and in most cases 1 week of temperature acclimation was adequate. Stocks of fish responded differently after 11 days of bioassay, although differences were not detected after 4 days of bioassay.

ADELMAN, I. R., AND L. L. SMITH, Jr. 1972. Toxicity of hydrogen sulfide to goldfish (*Carassius auratus*) as influenced by temperature, oxygen, and bioassay techniques. J. Fish. Res. Bd. Canada 29: 1309–1317.

Les auteurs ont effectué des essais biologiques dans le but d'évaluer les effets de la température et de l'oxygène sur la toxicité de $H_2S$ à l'endroit du cyprin doré (*Carassius auratus* L.) et d'examiner certains facteurs susceptibles d'affecter les résultats d'essais biologiques. Le rapport entre la toxicité de $H_2S$ et la température est négativement logarithmique entre 6.5 et 25 C. Le TL50 de 96 h moyen est de 530 μg/litre à 6 C et de 4 μg/litre à 25 C. Aux températures de 14, 20 et 26 C, le plus gros des mortalités par $H_2S$ prend fin après 11 jours, et les TL50 de 11 jours à ces températures diffèrent de façon significative. Au cours d'essais biologiques avec et sans acclimatation préalable à l'oxygène, une diminution des teneurs en $O_2$ augmente la toxicité de $H_2S$. Dans le premier cas, les TL50 moyens sont de 62 et 48 μg/litre de $H_2S$ à des teneurs en $O_2$ de 6 et 1.5 mg/litre respectivement. Dans le deuxième cas, ces valeurs sont de 71 et 53 μg/litre aux mêmes teneurs en $O_2$. La variabilité des résultats des essais n'est pas affectée par des températures de 14, 20 et 26 C et, dans la plupart des cas, une acclimatation d'une semaine à la température est suffisante. Différents stocks de poissons réagissent différemment après 11 jours d'essais, quoique ces différences n'apparaissent qu'après 4 jours d'essais.

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HYDROGEN sulfide may occur naturally in many lakes during the summer (Hutchinson 1957; Bonn and Follis 1967), in ice-covered lakes (Scidmore 1957), and at the sediment–water interface in fish spawning marshes (Adelman MS 1969). In these instances it is produced by the anaerobic decomposition of organic material in the sediments. Hydrogen sulfide is also produced by the decomposition of organic effluents from municipal sewage and many industries, particularly wood fiber sludge from pulp mills (Colby and Smith 1967; Ziebell et al. 1970). It may also be released directly in industrial effluents from pulp mills (Van Horn et al. 1949), from oil refineries (Dorris et al. 1960), and from chemical and gas manufacturers (Ellis 1937).

Adelman and Smith (1970) reviewed the literature on the toxic effects of $H_2S$ to fish. Recently, Smith and Oseid (1972) reported 96-hr TL50 values for

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eggs and sac fry of various fish species. The range for eggs was from 28 to 87 µg/liter $H_2S$ and for sac fry from 7 to 26 µg/liter. Oseid and Smith (1972) found the swimming endurance of bluegill sunfish (*Lepomis macrochirus*) was reduced after chronic exposure to 0.4 µg/liter $H_2S$. Little information is available from the literature on the effects of temperature or oxygen concentrations on $H_2S$ toxicity. Shelford (1917), working with the orangespotted sunfish (*Lepomis humilis*), and Colby and Smith (1967), working with *Gammarus pseudolimnaeus* and walleye pike (*Stizostedion vitreum*) eggs, found that a decrease in dissolved-oxygen concentration increased the toxicity of sulfide solutions. Adelman and Smith (1970) found no increase in mortality of northern pike (*Esox lucius*) eggs from $H_2S$ with decreased oxygen concentrations, but did find an increase in mortality of sac fry.

Temperature and dissolved-oxygen concentrations have been demonstrated to affect the toxicity of most poisons by many workers. Since low oxygen concentrations are usually found when $H_2S$ is present and since $H_2S$ may be found under a variety of temperature conditions, the present study is directed at determination of the effect of these two environmental variables on the acute toxicity of $H_2S$ to the goldfish (*Carassius auratus*).

The consistency of biossay results may be influenced by many factors occurring prior to and during the bioassay (Sprague 1969, 1970). There has been an attempt in recent years to standardize bioassay techniques so that research by different investigators will be more comparable. In connection with the examination of the effect of temperature and oxygen on $H_2S$ toxicity, the influence of these factors and a number of others on variations in bioassay results were investigated. These factors included: (1) acclimation to low oxygen concentrations prior to testing the effect of oxygen on $H_2S$ toxicity; (2) the length of temperature acclimation time prior to testing; (3) the test temperature; (4) different stocks of fish obtained from the same source at different intervals throughout 1 year.

## Materials and Methods

### Acute Bioassays at Various Temperatures

Bioassays were conducted in two identical continuous-flow units in which gaseous $H_2S$ was mixed with well water and dilutions of the water distributed to the test chambers. This apparatus is as described by Colby and Smith (1967), with modifications by Adelman and Smith (1970). Each unit included six 25-liter test chambers (one control and five treatments), 40 × 25 × 28 cm deep, which were constructed of glass and G.E. Silicone Seal. With a flow rate of 280 ml/min, 90% replacement of water occurred in approximately 1½ hr.

Two stocks of goldfish from Ozark Fisheries, Inc., Stoutland, Mo., and three stocks from the Federal hatchery at Lake Mills, Wisc., were tested at various times during a 16-month period. Variation in fish age in different bioassays was from approximately 4 months to 2½ years, and the range in mean weights was from 1.40 to 14.63 g. Within any bioassay the age, weight, and stock of fish was the same.

Fish from both sources carried skin flukes, *Gyrodactylus* sp., and all were treated with 0.86 mg/liter methylene blue for 2 days with an additional 0.86 mg/liter added on the third day. The fish were held in the solution for a total of 5 days. This treatment reduced incidence to a low level but did not eliminate it. Fish held in the laboratory were re-treated periodically when the incidence of flukes increased. Fish were not used for bioassay until at least 2 weeks after treatment.

One week prior to a bioassay 65 fish from each stock to be tested were removed from holding tanks where temperatures ranged from 10 to 13 C and placed in a 38-liter acclimating aquarium in a water bath. Oxygen concentration in the aquarium was maintained near saturation and a continuous flow of water passed through it. Temperatures were raised or lowered from that of the holding room to the desired bioassay temperature at the rate of 4 C/day, which insured exposure to the test temperature for at least 3 days. In bioassays conducted at the highest or lowest temperatures, fish may not have been fully acclimated. However, results described in succeeding sections indicate that this was an acceptable acclimation regime at least for increasing temperatures. The fish were fed Oregon moist pelletted trout food until 1 day prior to the bioassay. In each bioassay 10 fish were randomly assigned to each chamber. Hydrogen sulfide was then raised to the desired concentration within a period of 3 hr. Mortality was recorded at 24-hr intervals.

Water for all bioassays was from a deep well (Table 1). During the tests, dissolved oxygen was measured with a polarographic oxygen meter, and *p*H with a meter. Dissolved sulfide was determined colorimetrically (American Public Health Association et al. 1971). The $H_2S$ concentration was calculated from dissolved sulfide and *p*H after making adjustments for the temperature and ionic strength (Pomeroy 1941).[1]

The dissolved oxygen, temperature, *p*H, and total alkalinity were measured once a day and the $H_2S$ at least twice a day and frequently more often. On the total of 29 bioassays at various temperatures, mean dissolved oxygen concentration was 5.84 mg/liter (range, 4.83–7.08; standard deviation, 0.68), mean *p*H was 7.80 (range, 7.63–7.99; standard deviation, 0.097), and mean total alkalinity was 237 mg/liter (range, 225–262; standard deviation, 7.3).

Median tolerance limits (TL50) were estimated for each bioassay by the graphical method (American Public Health Association et al. 1971). In some instances, 0 and/or 100% survival points were used in the estimation.

[1]The data were accumulated before the new ionization constants were reported by the American Public Health Association et al. (1971). Multiplying the reported $H_2S$ values by a factor of 1.47 will closely approximate values computed with the new ionization constants.

TABLE 1. Analysis of well water used in bioassays.[a]

| Item | Concn (*mg/liter*) |
|---|---|
| Total hardness as $CaCO_3$ | 220 |
| Calcium as $CaCO_3$ | 140 |
| Magnesium as $CaCO_3$ | 70 |
| Iron | 0.02 |
| Manganese | 0.04 |
| Chloride | <1.0 |
| Sulfate | <5 |
| Fluoride | 0.22 |
| Total phosphorus | 0.03 |
| Sodium | 6 |
| Potassium | 2 |
| Ammonia nitrogen | 0.20 |
| Organic nitrogen | 0.20 |
| Phenols | <0.005 |
| Cu, Cd, Zn, Ni, Pb, Hg | <0.01 |

[a]Water taken from well head after iron removal and before aeration and heating.

### ACUTE BIOASSAYS AT VARIOUS OXYGEN CONCENTRATIONS

The same two units were used for bioassays with different oxygen levels as for those with different temperatures. The test chambers consisted of six acrylic plastic egg-hatching jars (depth, 45 cm; diameter, 14.5 cm; volume of water, 6.1 liters). The smaller test chambers permitted precise oxygen control and 90% water replacement in approximately 50 min.

Five stocks of goldfish from Ozark Fisheries, Inc., all hatched during May and June 1970, were used for bioassays. Age of fish ranged from 6 months in the earliest to 17 months in the final bioassays, and mean weights from 3.94 to 6.06 g. During the holding period, skin flukes were treated for 1 hour on 2 consecutive days with 0.25 ml/liter of formalin, but 100% erradication was rarely achieved. Bioassays were conducted not less than 10 days after treatment.

Tests were conducted in pairs with a different oxygen concentration in each of the two. One week before the start of a test, 100 fish were divided between two 38-liter aquaria for acclimation. Oxygen was held at saturation when temperature acclimation only was required, and water flow was continuous. Acclimation to specified oxygen concentrations was achieved by continuous withdrawal of water from the appropriate level of a reaeration system similar to that described by Brungs (1971). Since well water entered the laboratory with approximately 0.5 mg/liter oxygen, degassing before aeration was not necessary. Oxygen concentrations were adjusted to the desired level at intervals over 24 hr, and temperature was raised to 17.5 C in 2 days for both groups.

At the start of a pair of bioassays, fish were randomly assigned, eight per chamber, to the five treatment and one control chambers in which the desired oxygen and $H_2S$ concentrations had previously been set. The bioassay procedures and calculations of TL50 values were the same as for the temperature experiments except that oxygen concentrations were measured more frequently. The mean temperature for the 21 bioassays was 17.46 C (range, 17.0–17.8; standard deviation, 0.21), mean *p*H was 7.69 (range, 7.64–7.80; standard deviation, 0.036), and mean total alkalinity was 229 mg/liter (range, 205–245; standard deviation, 8.3).

### TESTS OF BIOASSAY VARIABILITY

Twenty-one bioassays were conducted during a 1-year period, using three diluters modified from that described by Mount and Brungs (1967) and Brungs and Mount (1970). Each time the diluter cycled, the incoming water tripped a "dipping bird" dispenser to release a measured volume of sodium sulfide stock solution directly into each treatment chamber. The four treatment and one control chambers in each diluter were constructed of glass and G.E. Silicone Seal, measured 50 × 25 × 20 cm, and contained 20 liters. With a flow rate of 445 ml/min, 90% replacement of water occurred in approximately 1¾ hr. The *p*H was controlled by dispensing a sulfuric acid solution into the head tank water supply with a "dipping bird," and the temperature was controlled by a hot water heat-exchange system in the head tank.

The entire experiment was conducted in a 3 × 3 factorial design with three temperatures (14, 20, 26 C), three acclimation times (1, 3, and 5 weeks), and seven blocks (stocks of fish). Approximately every 8 weeks a new shipment of goldfish was obtained from Ozark Fisheries. Six of these stocks were hatched in May and June 1970 and one in May 1971. Their age at time of bioassay ranged from 6 to 15 months, and mean weights ranged from 3.17 to 5.70 g. On arrival in the laboratory the temperature was lowered from that of the shipment water (15–23 C) to the holding tank temperature (10–13 C) within 24 hr, and on the second and third day after arrival the formalin treatment described above was applied. Three days later, 500 of the fish were divided into three groups, and each group was placed in a 400-liter acclimation tank. Vigorous aeration circulated the water around polyethylene coils of a hot water heat exchanger immersed in each tank, and the temperature was raised to the desired levels of 14, 20, and 26 C at the rate of 5 C/day.

After 1, 3, and 5 weeks from the start of acclimation, approximately one-third of the stock in each tank was removed for an 11-day bioassay of $H_2S$ at the acclimation temperature. The fish from each group were randomly assorted into the four treatment and one control chambers of the appropriate diluter and were not fed during the entire bioassay. The bioassays were conducted in the same manner as those described previously, and the same well water was used (Table 1). Means and standard deviations for temperature *p*H, oxygen, and total alkalinity (TA) were as follows:

| | Diluter 1 | Diluter 2 | Diluter 3 |
|---|---|---|---|
| Temp (*C*) | 14.11±0.12 | 20.05±0.12 | 25.95±0.10 |
| *p*H | 7.65±0.022 | 7.64±0.037 | 7.65±0.048 |
| Oxygen (*mg/liter*) | 8.04±0.43 | 7.96±0.40 | 7.56±0.31 |
| TA ($CaCO_3$) (*mg/liter*) | 216.1±16.79 | 210.0±8.71 | 158.6±9.36 |

Hydrogen sulfide, *p*H, and temperature were measured once per day and dissolved oxygen once per week.

## Results and Discussion

### Acute Bioassays at Various Temperatures

The mean 96-hr TL50 ranged from 530 µg/liter at 6.5 C to 44 µg/liter at 25 C. The log of the TL50 increases proportionally to a decrease in the log of temperature (Fig. 1). The linear regression is described by the equation:

$$\log \hat{Y} = -1.8527 \log X + 4.2325$$

where $\hat{Y}$ is the 96-hr TL50 and X is the temperature. The regression is highly significant ($F_{1,25} = 254.86$, $P < .01$), and 91% of the variation in TL50 is attributable to temperature (Steel and Torrie 1960). The regression line for these goldfish is compared with that for fathead minnows, *Pimephales promelas*, (L. L. Smith, Jr. unpublished data) and with that for the uniform stocks of goldfish in the tests of biossay variability reported below (Fig. 2). The increase in toxicity of $H_2S$ with increasing temperatures is similar for the three groups with all goldfish more tolerant than the fatheads and the uniform stock of goldfish more tolerant than the mixed stocks. Since the three points defining the regression line for the uniform group are the means of 21 bioassays, they are more precise than any single point defining the regression for the mixed stock where a maximum of six bioassays were conducted at an individual temperature. The regression line for the uniform stock indicates that at least with this group the effect of temperature is slightly curvilinear on a logarithmic plot.

Since the relation of TL50 to temperature is logarithmic, increases in tolerance at the colder temperatures are larger than at higher temperatures. A 10 C change from 7 to 17 C increases toxicity about 5.5 times, but a 10 C increase from 15 to 25 C increases toxicity about 2.6 times. Sprague (1969) reviewed the literature concerning the effect of

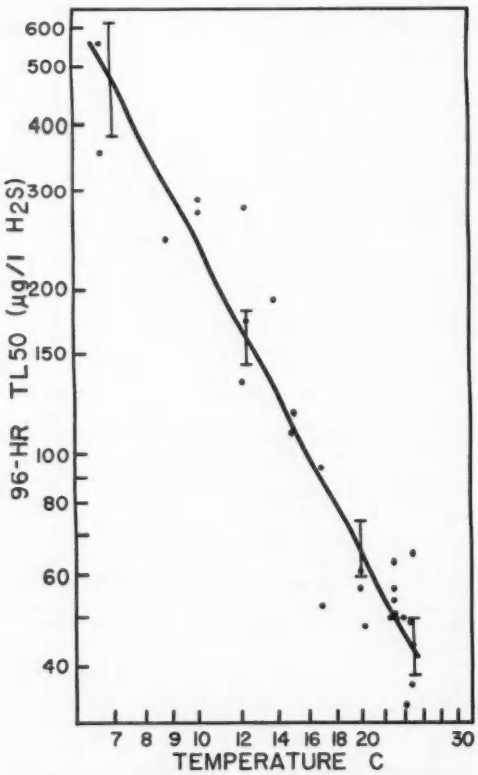


Fig. 1. The effect of temperature on the 96-hr TL50 of $H_2S$ to goldfish with 95% confidence limits.

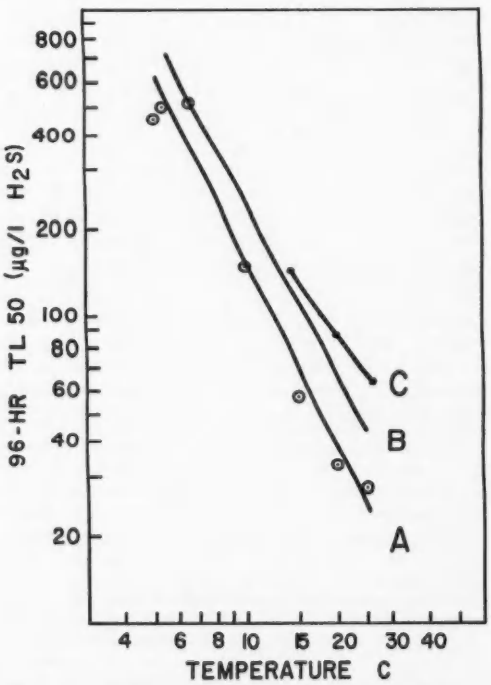


Fig. 2. The effect of temperature on the 96-hr TL50 of $H_2S$ to three groups of fish: (A) fathead minnows, (B) goldfish from bioassays on temperature effects, and (C) goldfish from bioassays for determination of variability.

temperature on acute fish mortality from various toxicants and noted that increased mortality with increased temperatures was quite common. A reverse effect has been reported for phenol and some pesticides. Increases in toxicity with higher temperatures must be evaluated on the basis of exposure time. Sprague (1969) points out that at lower temperatures a slower mortality rate early in a bioassay will not always result in an overall decrease in toxicity. Lloyd (1960) demonstrated that toxicity of zinc in short time intervals was greater at higher temperatures, but in 3 days there was no difference between temperatures of 13.5 and 21.5 C. The relation of $H_2S$ toxicity to temperature expressed in Fig. 2 is for 96 hr. In the 11-day bioassays reported below, the differences in TL50 values between tests conducted at 14, 20, and 26 C decreased with time, but there was a significant difference between each temperature at every time interval (Fig. 3). At 11 days the toxicity curves at each temperature appear to have reached an asymptote, implying little subsequent change. These data indicate that there is an actual decrease in toxicity of $H_2S$ at colder temperatures as well as a slower rate of mortality.

## Acute Bioassays at Various Oxygen Concentrations

In bioassays without acclimation, the mean TL50 was 71 µg/liter $H_2S$ at 6.0 mg/liter oxygen and 53 µg/liter $H_2S$ at 1.5 mg/liter oxygen. In tests with acclimation, the mean TL50's were 62 and 48 µg/liter $H_2S$ at the same oxygen concentrations. In eight of 10 pairs the bioassay conducted at the lower oxygen concentration resulted in a lower 96-hr TL50 (Table 2). This result indicates that decreased oxygen concentrations increase $H_2S$ toxicity. Shelford (1917), Colby and Smith (1967), and Adelman and Smith (1970) found this same effect in other species, although the latter authors found no effect of oxygen differences on toxicity of $H_2S$ to northern pike eggs. Sprague (1969) reviewed the work of others who found similar effects with other toxicants. In two bioassays where the fish were acclimated to the oxygen concentration of the bioassay, the 96-hr TL50's were higher than all but one in the unacclimated series. If these two values are eliminated, the relation of the 96-hr TL50 to the oxygen concentration forms the significant linear regression ($F_{1,8} = 9.78$, $P < .05$) (Steel and Torrie 1960):

$$\hat{Y} = 2.83\,x + 44.28$$

where $\hat{Y}$ is the 96-hr TL50 and $x$ is the oxygen concentration (Fig. 4). The linear regression from bio-

Table 2. The effect of oxygen on $H_2S$ toxicity in paired bioassays.

| Bioassay no. | Mean acclimation oxygen (*mg/liter*) | Mean oxygen in bioassay (*mg/liter*) | 96-hr TL50 (*mg/liter* $H_2S$) |
|---|---|---|---|
| | *With prior oxygen acclimation* | | |
| 1A | 2.88 | 3.41 | 51 |
| 1B | 4.83 | 4.97 | 58 |
| 2A | 2.13 | 1.96 | 58 |
| 2B | 6.07 | 6.13 | 63 |
| 3A | 1.25 | 1.05 | 70 |
| 3B | 4.16 | 4.36 | 54 |
| 4A | 3.08 | 3.07 | 55 |
| 4B | 6.55 | 6.13 | 66 |
| 10A | 1.39 | 1.00 | 95 |
| 10B | 4.06 | 4.16 | 49 |
| 12A | 1.50 | 1.40 | 46 |
| 12B | 2.84 | 2.29 | 50 |
| | *Without prior oxygen acclimation* | | |
| 5A | Sat[a] | 1.18 | 44 |
| 5B | " | 4.28 | 53 |
| 6A | " | 1.95 | 55 |
| 6B[b] | | | |
| 7A | " | 1.81 | 49 |
| 7B | " | 5.72 | 80 |
| 8A | " | 2.81 | 66 |
| 8B | " | 6.31 | 69 |
| 9A | " | 1.08 | 60 |
| 9B | " | 4.83 | 65 |

[a]Saturation. dissolved oxygen varied from approximately 9–11 mg/liter.
[b]Bioassay not completed due to apparatus failure.

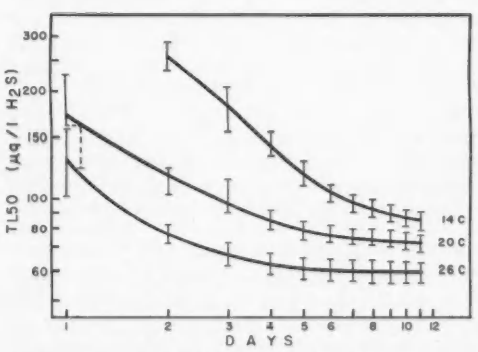


Fig. 3. Changes in TL50 values during 11 days exposure to $H_2S$ at three temperatures with 95% confidence limits.

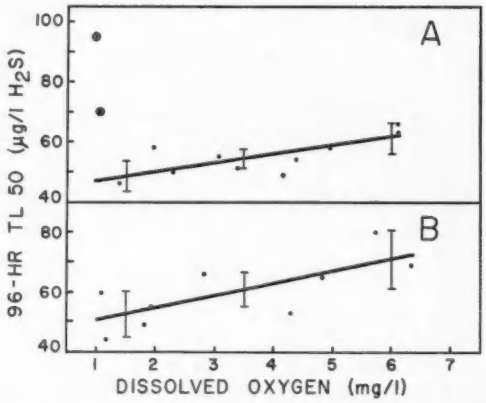


FIG. 4. The effect of oxygen on the 96-hr TL50 of $H_2S$: (A) with oxygen acclimation prior to the bioassay and (B) without prior oxygen acclimation. The circled points are not included in the regression calculation, and 95% confidence limits are spaced at uniform intervals.

assays without oxygen acclimation is also significant ($F_{1,7} = 8.09$, $P < .05$):

$$\hat{Y} = 4.08x + 46.52$$

where $\hat{Y}$ and $x$ are the same as above (Fig. 4). There is a considerable variation among points around both regression lines since in the former only 55% and in the latter 54% of the variation in the 96-hr TL50 is attributable to oxygen concentration. The rate of increase in $H_2S$ toxicity with decreasing oxygen is the same with or without prior acclimation as was shown by an analysis of covariance ($F_{1,15} = .52$, not significant at .05) (Snedecor and Cochran 1967).

This analysis also indicated that the elevation of the regression line for bioassays without acclimation was significantly higher than that for bioassays with acclimation ($F_{1,6} = 5.23$, $P < .05$). Acclimation to oxygen prior to testing does not increase goldfish resistance to acute $H_2S$ toxicity and, in fact, appears to make them more sensitive. This result is the opposite of that of Lloyd (1960), who found that rainbow trout acclimated to low oxygen for 18 hr were more resistant to zinc than unacclimated fish. Use of different stocks of goldfish may have contributed at least in part to the unexpected effect. Since the actual difference in elevation of the regression lines is small, and since there is a great variation in individual points, acclimation to oxygen does not affect $H_2S$ toxicity appreciably, except for a possible effect at very low oxygen concentrations.

The two high TL50's at the low oxygen concentrations may be due to changes in the fish's metabolism through acclimation. Kutty (1968) reported anaerobic metabolism in goldfish at low oxygen concentrations. The mode of toxic action of $H_2S$ in fish has not been investigated, but in humans at high concentrations it immediately reduces the oxyhemoglobin, and at lower concentrations it inactivates respiratory enzymes, primarily the cytochrome system (Goodman and Gilman 1955). If fish are metabolizing anaerobically, the $H_2S$ may be poisoning an enzyme system that the fish are not using to any extent at that time. This increased resistance to $H_2S$ at very low oxygen concentrations was not observed in unacclimated fish. Since Kutty (1968) has shown that at low oxygen, anaerobic metabolism will occur with or without acclimation, a period of adjustment may be required before the increased resistance to $H_2S$ occurs.

### TESTS OF BIOASSAY VARIABILITY

The 3 × 3 factorial design of the experiment in variability permitted analysis for differences due to acclimation time, temperature, and fish stocks. Since the *p*H and fish size may affect the toxicity of $H_2S$ and could not be held precisely constant in different bioassays, an analysis of covariance (Snedecor and Cochran 1967) with *p*H and weight of the fish as covariates was used for both the 4- and 11-day TL50 values (Table 3).

There was a significant difference in 4- and 11-day TL50 values in tests conducted at different temperatures: $F_{2,43} = 167.27$, $P < .01$ for the former and $F_{2,44} = 39.23$, $P < .01$ for the latter (Fig. 3). No significant difference in the 4-or 11-day TL50 values was noted in bioassays conducted after 1, 3, or 5 weeks of temperature acclimation. With the 4-day TL50's, $F_{2,43} = 2.56$, not significant at .05; the 11-day TL50's, $F_{2,44} = .76$ not significant at .05. Brett (1946) showed that acclimation of goldfish to increased temperature as measured by their thermal tolerance proceeded at different rates depending on the range in which the increase occurred. An increase from 4 to 12 C required 20 days for acclimation to the higher temperature whereas the same increase from 20 to 28 C required 3 days. An increase from 12 to 20 C required 7 days of acclimation. This rise is similar to that in one group of fish of the present study where temperature was increased from approximately 12 to 20 C. Since these goldfish should require 7 days to acclimate in accordance with Brett's data, it might be expected that those fish raised from 12 to 26 C would take longer with a resulting increase in TL50's after the third or fifth week of acclimation. Fish stocks 1, 2,

TABLE 3. The effect of temperature, acclimation time, and fish stock on the 96-hr and 11-day TL50 (*mg/liter* $H_2S$).[a]

| Weeks of acclimation | Fish stock | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | | | *14 C* | | | | |
| 1 | 120 | 81 | 80 | 82 | 94 | 80 | 73 |
| | (162) | (125) | (156) | (142) | (124) | (118) | (129) |
| 3 | 85 | 78 | 79 | 79 | 103 | 87 | 75 |
| | (126) | (150) | (132) | (163) | (195) | (152) | (180) |
| 5 | 82 | 68 | 66 | 80 | 96 | 99 | 76 |
| | (163) | (140) | (132) | (134) | (155) | (150) | (112) |
| | | | *20 C* | | | | |
| 1 | 76 | 65 | 77 | 78 | 75 | 57 | 66 |
| | (81) | (76) | (80) | (83) | (82) | (86) | (68) |
| 3 | 79 | 74 | 73 | 84 | 67 | 55 | 52 |
| | (115) | (98) | (89) | (92) | (81) | (68) | (71) |
| 5 | 81 | 76 | 72 | 79 | 84 | 61 | 69 |
| | (87) | (82) | (77) | (88) | (94) | (76) | (76) |
| | | | *26 C* | | | | |
| 1 | 54 | 52 | 56 | 55 | 62 | 64 | 50 |
| | (54) | (57) | (58) | (64) | (76) | (71) | (50) |
| 3 | 65 | 52 | 51 | 61 | 60 | 84 | 46 |
| | (71) | (52) | (53) | (63) | (64) | (84) | (55) |
| 5 | 63 | 63 | 65 | 56 | 66 | 72 | 55 |
| | (66) | (64) | (68) | (57) | (66) | (72) | (59) |

[a]96-hr TL50 in parentheses.

and 3 tested at 26 C become more tolerant to $H_2S$ after 3 or 5 weeks of acclimation, but stocks 4–7 do not show this trend (Table 3). Stocks 4–7, obtained in April–September, experienced seasonal mean temperatures ranging from approximately 12 to 26 C in the supplier's holding pond, and stocks 2 and 3, obtained in January and February, were exposed to pond temperatures of about 4–7 C. This exposure to cold temperatures may have resulted in a longer acclimation time in the experiments. Stock 1, obtained in November, was exposed to pond temperatures of about 13 C. This stock would be expected to respond as stocks 4–7 with no increased acclimation time, but increased time was required.

Sprague (1969) indicates metabolic acclimation might be more important and proceed at a different rate than acclimation as judged by lethal temperatures. Peterson and Anderson (1969) concluded that complete metabolic acclimation of Atlantic salmon to temperature change required about 2 weeks. The goldfish in the present study required 1 week or less with two or three possible exceptions, if toxicity resistance is indicative of metabolic acclimation.

The analysis of covariance indicated that there was no significant difference between the 4-day TL50 values for the seven stocks of fish ($F_{6,43} = 1.57$, not significant at .05), but there was a significant difference between the 11-day TL50 values ($F_{6,44} = 4.98$, $P < .01$). Stocks that are different in their overall resistance to a toxicant after 11 days may have similar rates of mortality during the earlier period of bioassay. Handling and sudden exposure to the toxicant may have increased the initial rates of mortality sufficiently in these bioassays to mask differences between stocks. If this stress masked actual effects, prolonged tests may be required to show real differences.

To test the effect of temperature on the variability of the results, the coefficient of variability was computed from the 4-and 11-day TL50's for each temperature. At 14, 20, and 26 C the coefficient of the variability of the 4-day TL50 was 14, 13, and 14%,

respectively, and of the 11-day TL50, 15, 13, and 14%, respectively, indicating no essential difference in the variability due to temperatures.

## Conclusions

The greatly increased resistance of goldfish to $H_2S$ at lower temperatures is probably of adaptive advantage. In the summer, naturally occurring $H_2S$ is generally restricted to the hypolimnion of lakes; thus, fish may escape to the upper water strata. However, in shallow, ice-covered lakes $H_2S$ may be found at most depths and may contribute to winter fish kills (Scidmore 1957). Under these conditions goldfish could survive higher $H_2S$ concentrations than in the summer when escape would be possible. If $H_2S$ persists for a long period during the winter, deleterious sublethal effects may occur, but present data does not permit any predictions on effects of temperature.

Since $H_2S$ usually occurs only at relatively low oxygen concentrations, its toxicity at these levels may have important environmental significance. However, the rate of increased toxicity with lowered oxygen is not very great, with the 96-hr TL50 reduced only about 26% between oxygen concentrations of 6 and 1 mg/liter. Furthermore, the difference in $H_2S$ concentrations between bioassay chambers where all or none of the fish died was small. A mean $H_2S$ concentration of 87 µg/liter caused 100% mortality and a mean of 36 µg/liter caused 0% mortality where 0 and 100% response occurred. Since complete mortality or survival occurs within a narrow range of $H_2S$, and since the rate of decreased resistance with lowered oxygen is small, the oxygen concentration will not have an important effect on $H_2S$ toxicity in the environment. When $H_2S$ concentrations are greater than about 36 µg/liter at 17.5 C, some goldfish mortality will probably occur regardless of the oxygen concentration.

The analysis of factors that could affect bioassay variability shows that prior acclimation of goldfish to the oxygen concentration of the bioassay had little effect on acute $H_2S$ toxicity except at very low oxygen concentrations. One week of acclimation to temperatures of 14, 20, and 26 C appeared adequate. However, goldfish that experience different thermal histories than the stocks tested may require a longer time to acclimate. Fish held in winter conditions and tested at high temperatures and fish held at summer conditions and tested at low temperatures should be acclimated for a longer period of time.

The toxicity of $H_2S$ to different stocks of fish was not the same although early mortality rates were similar. It is believed that this difference may relate to the season of year rather than genetic differences. Hoar (1955) found greater tolerance to cold temperature of goldfish tested in the winter and greater tolerance to high temperature of fish tested in the summer. This effect was attributed to adaptive physiology rather than fat storage. Since seasonal changes in physiology do occur in fishes, differences in tolerance to toxicants might be expected.

Although the range in 4- and 11-day TL50's was greater at colder temperatures, the coefficient of variability was essentially the same at 14, 20, and 26 C. Therefore, variability of bioassay results is not affected by temperature in that range.

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ADELMAN, I. R. MS 1969. Survival and growth of northern pike (*Esox lucius* L.) in relation to water quality. Ph.D. Thesis. Univ. Minnesota, St. Paul, Minn. 195 p.

ADELMAN, I. R., AND L. L. SMITH, Jr. 1970. Effect of hydrogen sulfide on northern pike eggs and sac fry. Trans. Amer. Fish. Soc. 99: 501–509.

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1971. Standard methods for the examination of water and wastewater. 13th ed. New York, N.Y. 874 p.

BONN, E. W., AND B. J. FOLLIS. 1967. Effects of hydrogen sulfide on channel catfish (*Ictalurus punctatus*) Trans. Amer. Fish. Soc. 96: 31–37.

BRETT, J. R. 1946. Rate of gain of heat tolerance in goldfish. Univ. Toronto Stud. Biol. Ser. 53: 5–28.

BRUNGS, W. A. 1971. Chronic effects of low dissolved oxygen concentrations on the fathead minnow (*Pimephales promelas*). J. Fish. Res. Bd. Canada 28: 1119–1123.

BRUNGS, W. A., AND D. I. MOUNT. 1970. A water delivery system for small fish-holding tanks. Trans. Amer. Fish. Soc. 99: 799–802.

COLBY, P. J., AND L. L. SMITH, Jr. 1967. Survival of walleye eggs and fry on paper fiber sludge deposits in Rainy River, Minnesota. Trans. Amer. Fish. Soc. 96: 278–296.

DORRIS, T. C., W. GOULD, AND C. R. JENKINS. 1960. Toxicity bioassay of oil refinery effluents in Oklahoma, p. 276–285. *In* Biological problems in water pollution. Trans. 1959. Semin. R.A. Taft Sanit. Eng. Cent.

ELLIS, M. M. 1937. Detection and measurement of stream pollution. Bull. Bur. Fish. 48: 365–437.

GOODMAN, L. S., AND A. GILMAN. 1955. The pharmacological basis of therapeutics. 2nd. ed. Macmillan and Company Ltd., New York, N. Y. 1831 p.

HOAR, W. S. 1955. Seasonal variations in the resistance of goldfish to temperature. Trans. Roy. Soc. Can. 49: 25–34.

HUTCHINSON, G. E. 1957. A treatise on limnology. Vol. I. Geography, physics, and chemistry. John Wiley & Sons, Inc., New York, N.Y. 1015 p.

KUTTY, M. N. 1968. Respiratory quotients in goldfish and rainbow trout. J. Fish. Res. Bd. Canada 25: 1689–1728.

LLOYD, R. 1960. The toxicity of zinc sulfate to rainbow trout (*Salmo gairdnerii* Richardson). Ann. Appl. Biol. 48: 84–94.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21–29.

OSEID, D., AND L. L. SMITH, JR. 1972. Swimming endurance and resistance to copper and malathion of bluegills treated by long-term exposure to sublethal levels of hydrogen sulfide. Trans. Amer. Fish. Soc. (In press)

PETERSON, R. H., AND J. M. ANDERSON. 1969. Influence of temperature change on spontaneous locomotor activity and oxygen consumption of Atlantic salmon, *Salmo salar*, acclimated to two temperatures. J. Fish. Res. Bd. Canada 26: 93–109.

POMEROY, R. D. 1941. Hydrogen sulfide in sewage. Sewage Works J. 13: 498–505.

SCIDMORE, W. J. 1957. An investigation of carbon dioxide, ammonia, and hydrogen sulfide as factors contributing to fish kills in ice covered lakes. Prog. Fish-Cult. 19: 124–127.

SHELFORD, V. E. 1917. An experimental study of the effects of gas waste upon fishes, with special reference to stream pollution. Bull. I11. State Lab. Nat. Hist. 11: 380–412.

SMITH, L. L., JR., AND D. OSEID. 1972. Effects of hydrogen sulfide on fish eggs and fry. Water Res. (In press)

SNEDECOR, G. W., AND W. C. COCHRAN. 1967. Statistical methods. 6th ed. Iowa State Univ. Press, Ames, Iowa. 593 p.

SPRAGUE, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4: 3–32.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York, N.Y. 481 p.

VAN HORN, W. M., J. B. ANDERSON, AND M. KATZ. 1949. The effect of kraft pulp mill wastes on some aquatic organisms. Trans. Amer. Fish. Soc. 79: 55–63.

ZIEBELL, C. D., R. E. PINE, A. D. MILLS, AND R. K. CUNNINGHAM. 1970. Field toxicity studies and juvenile salmon distribution in Port Angeles Harbor, Washington. J. Water Pollut. Contr. Fed. 42: 229–236.

# Depth Distributions of Benthic Polychaetes in Two Fiords on Ellesmere Island, N.W.T.

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CURTIS, M. A. 1972. Depth distributions of benthic polychaetes in two fiords on Ellesmere Island, N.W.T. J. Fish. Res. Bd. Canada 29: 1319–1327.

The benthic fauna of Hare and Tanquary fiords was collected in replicate grab samples taken at standardized depths from 6 to 100 m. From this collection, 68 polychaete species were identified. Two of these, *Hartmania moorei* Pettibone and *Zeppelinia monostyla* (Zeppelin), have not previously been reported in the Arctic. Population densities of common species demonstrate a marked relation to depth and the distributions of single species were similar in each fiord. Distributions at less than 10 m in Tanquary Fiord appear to be greatly modified by the presence of fiord water, a brackish surface layer formed during the summer ice melt. Among the polychaetes, depth ranges and depths of greatest abundance usually differed and so the species appeared to be scattered along the depth gradient rather than grouped in distinct assemblages.

CURTIS, M. A. 1972. Depth distributions of benthic polychaetes in two fiords on Ellesmere Island, N.W.T. J. Fish. Res. Bd. Canada 29: 1319–1327.

L'auteur a étudié la faune benthique des fiords Hare et Tanquary à l'aide d'échantillonnages à la benne effectués en double à des profondeurs déterminées, entre 6 et 100 m. A même le matériel recueilli, il a identifié 68 espèces de polychètes dont deux, *Hartmania moorei* Pettibone et *Zeppelinia monostyla* (Zeppelin), n'avaient pas encore été signalées dans l'Arctique. Les espèces communes ont une densité de population nettement liée à la profondeur, et la répartition d'une espèce donnée est la même dans les deux fiords. A des profondeurs de moins de 10 m, l'eau de fiord, couche superficielle d'eau saumâtre formée par la fonte des glaces en été, semble influencer fortement la répartition dans le fiord Tanquary. La répartition des polychètes diffère généralement, quant à la gamme des profondeurs et la profondeur d'abondance maximale, de sorte que les espèces semblent dispersées le long du gradient de profondeur, plutôt que groupées en communautés distinctes.

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THE analysis of plant and animal distributions along environmental gradients promises to be a useful approach to community studies in ecology (Whittaker 1967; Mills 1969). There is an interesting opportunity to apply the technique of gradient analysis in arctic fiords, where relatively stable hydrographic conditions prevail at all depths throughout the year. In that region it is possible to examine the effects of changes in depth upon a benthic animal population without the necessity of dealing with corresponding changes in temperature, a problem characteristic of lower latitudes.

The overall pattern formed by the depth distributions of benthic animals is of special importance to the problems of community description. This is because the extent of grouping among species distributed along a depth gradient is a primary consideration in designating benthic communities. Yet, the followers of Petersen's approach to community description (Petersen 1913; reviewed by Thorson 1957) have largely neglected the details of depth distributions and, conversely, papers on depth distributions (Clark and Milne 1955; McIntyre and Eleftheriou 1968) have not referred to the theoretical aspects of community work. There has been difficulty in obtaining adequate data for relating the results from this type of gradient analysis to other community studies in benthic ecology. Hence, this paper presents a detailed account of the relation between depth and population density for a number of polychaete species commonly occurring in the Arctic.

## Methods

Hare and Tanquary fiords are branches of the Greely Fiord system of northern Ellesmere Island. Sampling stations were located halfway along the length of Hare Fiord, near van Hauen Pass (81°03′N, 86°20′W) and at

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the head of Tanquary Fiord (81°25′N, 76°55′W). The bottom topography of the area is rugged, with depths in excess of 200 m being found in both fiords. Fine glacial silt was encountered everywhere along the station transects, with some mixture of sand at depths less than 10 m.

The hydrographic conditions of the Greely Fiord system have been described by Ford and Hattersley-Smith (1965), and some additional records from Tanquary Fiord are given by Cairns (1967). Water temperatures in these high arctic fiords remain below or near 0 C throughout the year, and an ice cover that attains a thickness of 3 m is present from September to July. A surface layer of low-salinity water (fiord water) develops during the period of ice melt. This layer is ordinarily only a few meters thick but can, under certain circumstances, extend to greater depths. The tides of both fiords have a maximum amplitude of about 30 cm.

All bottom samples were collected with a 0.07 $m^2$ grab manufactured by Foerst (Chicago) and described by Ellis (1960). The grab was hauled with a hand-powered winch mounted either on an aluminum frame for work beside leads in the sea ice, or mounted in a freight canoe for work in open water. On the sea ice, precautions were taken to avoid sampling in a previously disturbed area by repeatedly moving the winch along the lead. The grab contents were sieved through a 1mm wire mesh and the samples preserved in formalin.

Nine or 10 samples were taken at each of the following depths, in transects running from the shore to deeper water: 6, 8, 10, 12, 15, 20, 25, 30, 40, 50, 60, 80, and 100 m. Replicate samples were obtained within ±2 m of the deeper standard depths and within ±1 m of the shallower standard depths. The Hare Fiord samples were taken during 1967 through two transverse leads located 1 km apart. At one lead, samples were obtained at standard depths of less than 40 m and at the other lead standard depths of 40 m and more were sampled. Because of melt conditions near shore, no samples were taken in Hare Fiord at depths of 6 and 10 m but samples at 8 and 12 m were obtained. Collections from Tanquary Fiord were taken from a freight canoe in 1967 along a single transect that included all standard depths to 100 m, excepting the 12 m depth.

## Results

### Taxonomic Results

From the collections in both fiords 68 species of polychaetes were identified (Table 1). A few of these species were found in additional samples not included in the depth-distribution analysis.

Many of these species are accounted for in Pettibone (1954, 1956, 1963); however, two of the species in the list have not previously been reported from the Arctic. A single specimen of *Hartmania moorei* Pettibone was collected at 10 m in Tanquary Fiord. The species was previously known only from the coast of New England (Pettibone 1963). A specimen regarded as *Zeppelinia monostyla* (Zeppelin) was found at 30 m in Hare Fiord. According to Fauvel (1923), *Zeppelinia monostyla* is known only from the saltwater aquaria of Fribourge en Brisgau.

There were some anomalies in species characteristics. Although *Prionospio cirrifera* has 6–11 short branchiae (Fauvel 1923), the specimens examined here possessed a single long pinnate pair, followed by five or six shorter pairs. The specimens of *Melinnexis annenkovae* correspond to Uschakov's account (1955) except that they had only three pairs of branchiae instead of four.

### Quantitative Results

Table 2 is a depth–frequency table giving the total numbers of specimens for each common polychaete species found at the different sample depths in Tanquary and Hare fiords. The figures in the tables represent total numbers of specimens from the 10 grab samples taken at each given depth. When fewer than 10 samples were available the actual total was extrapolated to arrive at an appropriate value for a hypothetical 10 samples.

Depth distributions of the more abundant polychaetes are further depicted in Fig. 1 through the fitting of polynomial curves by the least squares method to the original data. These curves are used to compare the distributions of single species in the two fiords and to illustrate the variety of depth-distribution patterns exhibited by different species. The order of polynomial equation used to generate each curve was selected pragmatically in that the lowest order curve to clearly illustrate the trend in the data was always utilized. The curves were discontinued when depths of relatively low population density were reached. Equations of greater than the fourth degree were never employed and second and third degree curves were adequate for all except the bimodal distributions.

Figure 2 presents a composite of the distributions for the common polychaete species in each of the two fiords by tracing changes in population density using percentages instead of absolute numbers. To produce this result, the curves in Fig. 1 have been transformed to percentages by assigning the value of 100% to the highest number generated by the polynomial equation for any sample depth.

## Discussion

### General Nature of the Distributions

It is evident from these results that polychaete distributions in the high arctic fiords are closely related to depth.

Most of the common polychaetes have similar depth distributions in both Hare and Tanquary

fiords. From Fig. 1, these species, collected abundantly in both fiords, illustrate this similarity of distribution patterns: *Aglaophamus malmgreni, Chaetozone setosa, Eteone flava, Laonice cirrata, Lumbrineris minuta, Lumbrineris tenuis, Lysippe labiata, Melinnexis annenkovae, Micronephthys minuta, Pholoe minuta,* and *Scoloplos armiger.* In Table 2 a number of less common species also appear to be most abundant at similar depths in both fiords. It seems reasonable to expect that similar results would be

TABLE 1. Polychaete species collected in Hare and Tanquary fiords.

| Species |
|---|
| Polynoidae |
| *Antinoella sarsi* (Malmgren) |
| *Antinoella badia* (Théel) |
| *Eucranta villosa* Malmgren |
| *Gattyana cirrosa* (Pallas) |
| *Hartmania moorei* Pettibone |
| *Melaenis loveni* Malmgren |
| Sigalionidae |
| *Pholoe minuta* (Fabricius) |
| Phyllodocidae |
| *Eteone flava* (Fabricius) |
| *Phyllodoce groenlandica* Oersted |
| Hesionidae |
| *Nereimyra punctata* (O. F. Müller) |
| Syllidae |
| *Syllis cornuta* Rathke |
| Nereidae |
| *Nereis zonata* Malmgren |
| Nephtyidae |
| *Aglaophamus malmgreni* (Théel) |
| *Micronephthys minuta* (Théel) |
| *Nephtys ciliata* (O. F. Müller) |
| Glyceridae |
| *Glycera capitata* Oersted |
| Lumbrineridae |
| *Lumbrineris fragilis* (O. F. Müller) |
| *Lumbrineris minuta* Théel |
| *Lumbrineris tenuis* (Verrill) |
| Sphaerodoridae |
| *Sphaerodorum gracilis* (Rathke) |
| *Ephesiella biserialis* (Berkeley and Berkeley) |
| Onuphidae |
| *Onuphis conchylega* M. Sars |
| Orbiniidae |
| *Scoloplos armiger* (O. F. Müller) |
| Apistobranchidae |
| *Apistobranchus tullbergi* (Théel) |
| Spionidae |
| *Laonice cirrata* (M. Sars) |
| *Malacoceros fuliginosus* (Claparède) |
| *Polydora caulleryi* Mesnil |
| *Prionospio cirrifera* Wiren |
| *Pygospio elegans* Claparède |
| *Spiophanes* sp. |
| Paraonidae |
| *Aricidea suecica* Eliason |
| Oweniidae |
| *Owenia fusiformis* Delle Chiaje |
| *Myriochele heeri* Malmgren |
| Cirratulidae |
| *Chaetozone setosa* Malmgren |
| *Cossura longocirrata* Webster and Benedict |
| *Zeppelinia monostyla* (Zeppelin) |
| Flabelligeridae |
| *Brada villosa* (Rathke) |
| Scalibregmidae |
| *Scalibregma inflatum* Rathke |
| *Polyphysia crassa* (Oersted) |
| Opheliidae |
| *Ammotrypane breviata* Ehlers |
| Capitellidae |
| *Notomastus latericeus* M. Sars |
| Dorvilleidae |
| *Stauronereis caecus* (Webster and Benedict) |
| Chaetopteridae |
| *Spiochaetopterus typicus* M. Sars |
| Maldanidae |
| *Leiochone polaris* (Théel) |
| *Maldane sarsi* Malmgren |
| *Praxillella affinis* (M. Sars) |
| Pectinariidae |
| *Pectinaria granulata* (Linné) |
| *Pectinaria hyperborea* (Malmgren) |
| Ampharetidae |
| *Amphicteis gunneri* (M. Sars) |
| *Glyphanostomum pallescens* (Théel) |
| *Lysippe labiata* Malmgren |
| *Melinna cristata* (M. Sars) |
| *Melinnexis annenkovae* Uschakov |
| *Sabellides borealis* M. Sars |
| *Sabellides octocirrata* M. Sars |
| Terebellidae |
| *Lanassa venusta* (Malm) |
| *Laphania boecki* Malmgren |
| *Leaena abranchiata* Malmgren |
| *Lysilla loveni* Malmgren |
| *Nicolea venustula* (Montagu) |
| *Polycirrus albicans* (Malmgren) |
| *Terebellides stroemii* (M. Sars) |
| Sabellidae |
| *Branchiomma infarcta* (Kröyer) |
| *Chone duneri* Malmgren |
| *Chone infundibuliformis* Kröyer |
| *Euchone analis* (Kröyer) |
| *Euchone papillosa* (M. Sars) |
| Serpulidae |
| *Spirorbis* sp. |

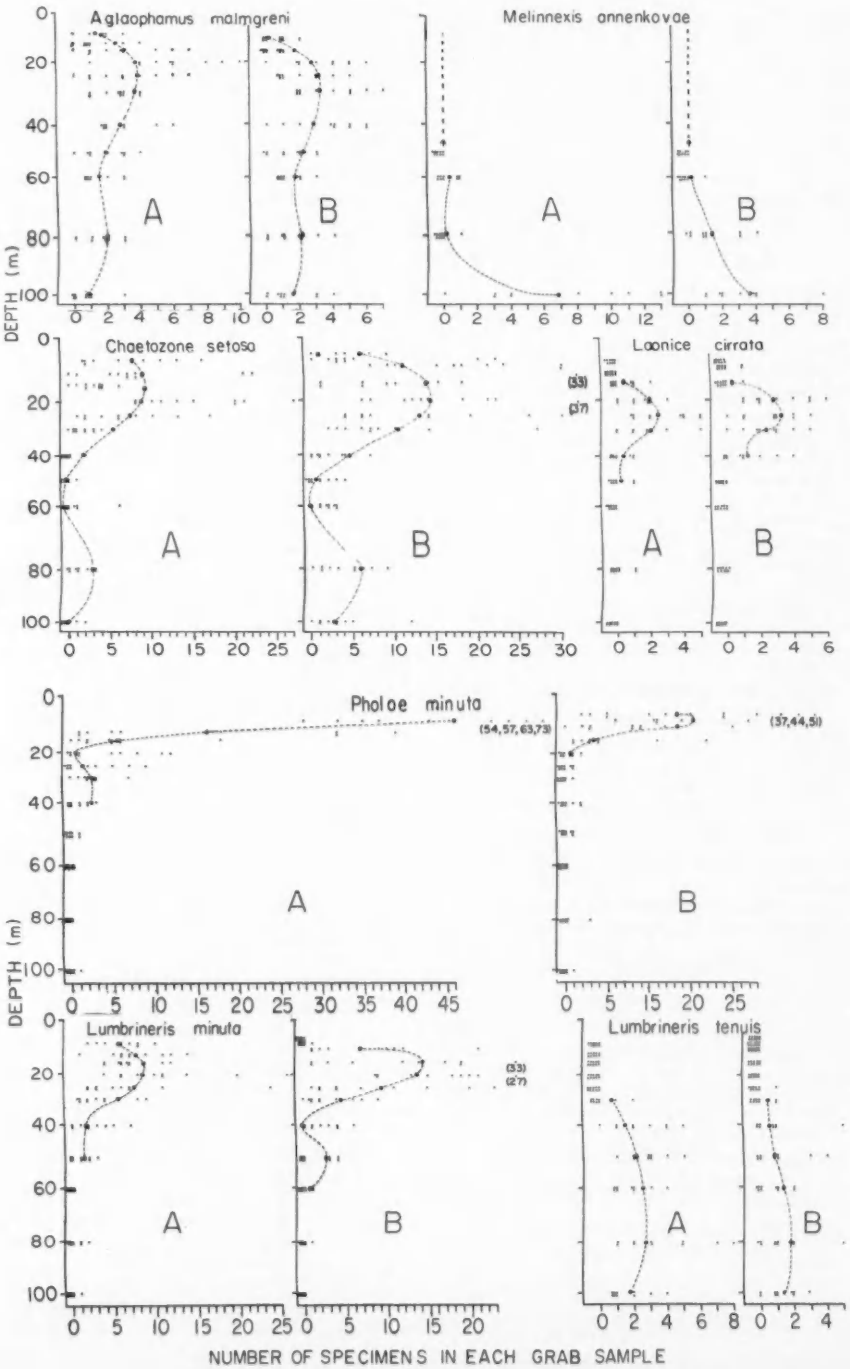
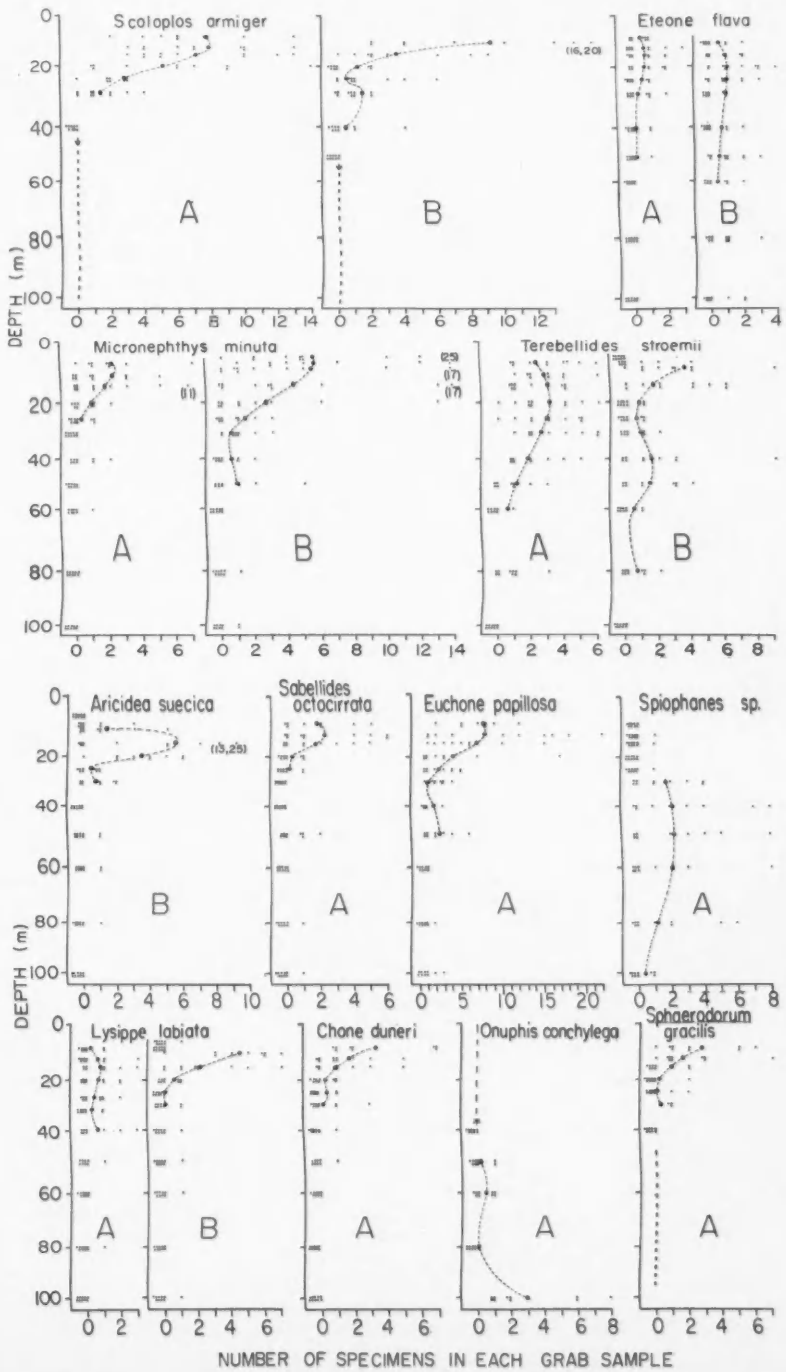



FIG. 1. Polynomial curves fitted to the depth-distribution data for common polychaete




species occurring in Hare (A) and Tanquary fiords (B). For explanation see text.

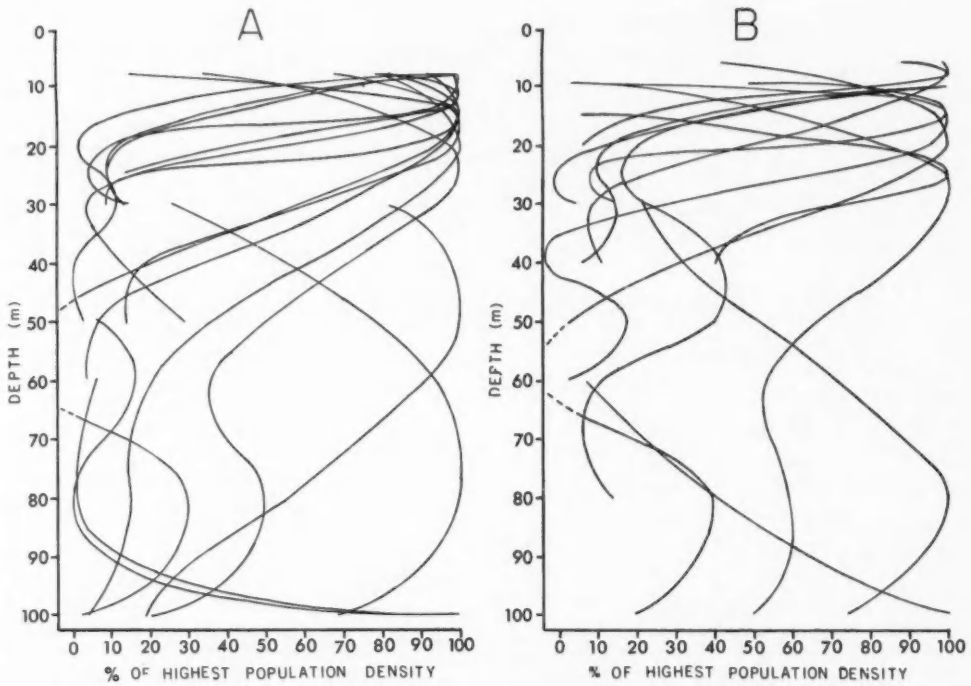



FIG. 2. A composite of depth distributions for common polychaetes in Hare (A) and Tanquary fiords (B). Here, the polynomial curves from Fig. 1 have been converted to percentage values relative to the highest population density generated by each curve.

obtained for other less abundant or widely ranged polychaetes if they could be collected in sufficient numbers for analysis.

The finding that depth distributions of individual species are much the same in these two sample areas, separated by over 300 km of sea bottom, strongly indicates that these same patterns are characteristic of other high arctic fiords having similar habitats. It is probable that the factors determining these distributions are of widespread importance in the Arctic.

Additional collections from Tanquary Fiord during 1967 and 1968 at several locations indicated that the depths of greatest population density are relatively stable and appear to vary only 5–10 m at the depths considered here (Curtis MS 1970).

The overall pattern of depth distributions in these fiords is best demonstrated by Fig. 2. The most significant feature of these composite figures is their illustration of how the greatest population densities of the various species are scattered along the depth gradient rather than clumped together at a few distinct depths. Although most of these species attain their highest numbers at depths of less than 30 m, their depth-distribution curves rarely coincide in peak or general configuration. This overall distribution pattern is much like that found by Whittaker (1967) for plant distributions as related to altitude and other environmental gradients. The implication is that species tend to be scattered along an environmental gradient rather than grouped in discrete assemblages. Mills (1969) postulated the existence of this pattern of distribution in benthic communities. Such a pattern cannot easily be dealt with by the traditional depiction of benthic communities (Petersen 1913) because of the complexity of individual species distributions along the depth gradient.

The factors responsible for this distribution pattern are unclear. Although the general pattern of scatter along environmental gradients can be attributed to niche diversification (Whittaker 1967), the factors determining individual species distributions must be dealt with more precisely. In fact, depth is an example of a "complex gradient" (Whittaker 1967) along which other factors change

TABLE 2. Total numbers of common polychaetes collected at each sample depth in Hare Fiord (A) and Tanquary Fiord (B). Figures were extrapolated when fewer than ten samples were obtained at a given depth.

| | | Depth (*m*) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | 8 | 10 | 12 | 15 | 20 | 25 | 30 | 40 | 50 | 60 | 80 | 100 |
| No. Samples | (A) | | 10 | | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 10 | 10 |
| No. Samples | (B) | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| *Aglaophamus malmgreni* | (A) | | 19 | | 14 | 30 | 59 | 32 | 27 | 31 | 22 | 15 | 18 | 9 |
| | (B) | – | – | 8 | | 5 | 33 | 22 | 38 | 38 | 14 | 14 | 21 | 15 |
| *Antinoella sarsi* | (A) | | 7 | | 1 | – | 2 | 1 | 3 | 1 | 3 | 3 | 2 | 3 |
| | (B) | 18 | 8 | 2 | | 1 | 5 | 2 | 1 | 3 | 3 | 3 | 6 | 6 |
| *Aricidea suecica* | (A) | | 2 | | 3 | 7 | 3 | 2 | 1 | 1 | 7 | 1 | 3 | – |
| | (B) | – | 6 | 13 | | 57 | 35 | 5 | 9 | – | 2 | 2 | 1 | – |
| *Chaetozone setosa* | (A) | | 65 | 85 | | 70 | 153 | 69 | 18 | 2 | 1 | 10 | 14 | 3 |
| | (B) | 33 | 80 | 180 | | 113 | 161 | 128 | 87 | 24 | 11 | 17 | 42 | 31 |
| *Chone duneri* | (A) | | 29 | | 14 | 14 | 2 | 2 | 5 | 1 | 1 | – | – | – |
| | (B) | 67 | 1 | 6 | | – | – | – | 1 | 6 | – | – | 1 | 1 |
| *Eteone flava* | (A) | | 6 | | 7 | 7 | 9 | 3 | 5 | 2 | 1 | – | – | – |
| | (B) | – | – | 4 | | 9 | 18 | 11 | 4 | 4 | 11 | 5 | 7 | 5 |
| *Euchone papillosa* | (A) | | 64 | | 101 | 49 | 33 | 18 | 12 | 6 | 13 | – | 1 | 3 |
| | (B) | 142 | 17 | 5 | | 4 | 6 | – | – | – | – | 1 | 1 | 1 |
| *Laonice cirrata* | (A) | | 1 | | 10 | 5 | 15 | 29 | 19 | 3 | 2 | – | 2 | – |
| | (B) | – | – | 2 | | 1 | 31 | 27 | 25 | 12 | – | – | – | – |
| *Lumbrineris minuta* | (A) | | 58 | | 78 | 70 | 116 | 70 | 35 | 28 | 10 | – | 4 | 1 |
| | (B) | – | 1 | 77 | | 125 | 160 | 89 | 33 | 21 | 15 | 2 | 1 | – |
| *Lumbrineris tenuis* | (A) | | – | | – | – | – | – | 4 | 25 | 22 | 19 | 38 | 17 |
| | (B) | – | – | – | | – | 1 | 1 | 2 | 10 | 11 | 7 | 22 | 13 |
| *Lysippe labiata* | (A) | | 3 | | 5 | 9 | 6 | 6 | 2 | 8 | 1 | – | 1 | – |
| | (B) | 1 | – | 44 | | 24 | 4 | – | 2 | 1 | 1 | 1 | – | 4 |
| *Melinnexis annenkovae* | (A) | | – | | – | – | – | – | – | – | – | 4 | 1 | 69 |
| | (B) | – | – | – | | – | – | – | – | – | – | 2 | 14 | 36 |
| *Nephtys ciliata* | (A) | | 3 | | 2 | 2 | 2 | 1 | – | – | – | – | – | – |
| | (B) | – | 2 | 3 | | 3 | 1 | – | – | – | – | – | – | – |
| *Nereimyra punctata* | (A) | | 2 | | 1 | – | – | 1 | – | – | – | – | – | – |
| | (B) | 38 | 20 | – | | – | – | – | – | – | – | – | – | – |
| *Onuphis conchylega* | (A) | | – | | – | – | – | – | – | – | 2 | 4 | – | 30 |
| | (B) | – | – | – | | – | – | – | – | – | 1 | – | – | 1 |
| *Pectinaria granulata* | (A) | | 10 | | 3 | 2 | 4 | – | – | – | – | – | – | – |
| | (B) | 98 | 59 | – | | – | – | – | – | – | – | – | – | – |
| *Pholoe minuta* | (A) | | 422 | | 153 | 50 | 44 | 26 | 26 | 10 | 2 | – | – | 1 |
| | (B) | 121 | 228 | 74 | | 50 | 6 | 3 | 1 | 5 | 3 | – | 3 | 1 |
| *Praxillella affinis* | (A) | | 4 | | 13 | 6 | – | – | – | – | 4 | 1 | – | – |
| | (B) | 2 | 12 | 1 | | – | – | – | – | – | 2 | – | – | – |
| *Prionospio cirrifera* | (A) | | 12 | | 3 | 2 | 1 | – | 10 | – | – | – | – | – |
| | (B) | 9 | 7 | 1 | | 4 | 13 | 6 | 2 | 22 | 8 | 1 | – | – |
| *Sabellides borealis* | (B) | 21 | 15 | 1 | | 3 | – | – | – | – | – | – | – | – |
| *Sabellides octocirrata* | (A) | | 18 | | 24 | 17 | 3 | 2 | 2 | – | 4 | – | 1 | 1 |
| *Scoloplos armiger* | (A) | | 76 | | 74 | 72 | 57 | 23 | 15 | – | – | – | – | – |
| | (B) | 15 | 12 | 91 | | 34 | 6 | 18 | 9 | 6 | 10 | – | 1 | – |
| *Sphaerodorum gracilis* | (A) | | 23 | | 21 | 5 | 2 | 3 | 3 | – | – | – | – | – |
| | (B) | – | – | 19 | | 10 | 1 | – | – | – | – | – | – | 1 |
| *Spiophanes* sp. | (A) | | – | | 1 | 1 | – | 1 | 15 | 28 | 22 | 13 | 16 | 3 |
| | (B) | – | – | – | | – | – | – | – | – | 3 | – | 5 | 5 |
| *Syllis cornuta* | (A) | | – | | – | – | – | – | – | – | – | – | – | 1 |
| | (B) | – | – | – | | – | – | 2 | 2 | 4 | 11 | 5 | 5 | 3 |
| *Terebellides stroemii* | (A) | | 26 | | 27 | 22 | 28 | 30 | 34 | 22 | 9 | 1 | 8 | – |
| | (B) | – | 8 | 30 | | 30 | 2 | 3 | 5 | 19 | 16 | 2 | 5 | – |

simultaneously; light, pressure, sediment, and microflora may all be involved to some degree. At this time it is not possible to relate any such factors to the distributions. There is a further possibility that interactions among the different benthic species operate directly in determining order along the depth gradient. Such biological factors should be of relatively greater influence in stable habitats, such as the arctic fiords, than in areas of more complex environmental conditions (Sanders 1968).

### The Effect of Fiord Water on the Distributions

Spärck (1933) and Thorson (1934) were the first to emphasize the importance of the brackish surface layer of fiord water in determining the distributions of some benthic animals in the Arctic when they considered its effect in establishing the limits of the "*Macoma calcarea* community" in East Greenland. Spärck defined fiord water as a surface water mass with a salinity of less than 25‰, formed by the melting sea ice and by the influx of fresh water from coastal rivers.

In Tanquary Fiord, Mr. H. Serson (Defence Research Board, Ottawa) related the depth of penetration of fiord water to wind patterns during 1967 in the sample area. He found (personal communication) that the fiord water was frequently wind-driven to the head of the fiord, where it then built up and pushed downward to displace the more saline water beneath it. In this manner the fiord water, from its usual depth of 2–5 m, penetrated to depths of almost 9 m. The halocline was abrupte and salinities of 15‰ were recorded at 8 m.

The fiord water has a marked effect upon polychaete distributions at the shallowest sample depths in Tanquary Fiord. This is shown in Table 2, where most of the common species in that fiord undergo an extensive change in numbers between the depths of 8 and 10 m. The population density of some species is greatly increased in going from 8 to 10 m, while the abundancies of other species are greatly reduced. Two separate explanations must be offered to account for the different directions of these changes in abundance.

*Aglaophamus malmgreni*, *Chaetozone setosa*, *Lumbrineris minuta*, *Scoloplos armiger*, *Sphaerodorum gracilis*, and *Terebellides stroemii* occur abundantly at depths of 10 m and more, but are present at much reduced numbers or totally absent at 6 and 8 m, the depths affected by fiord water. The absence of these species at shallower depths can be attributed to their inability to cope with the fluctuating hydrographic conditions there. In the sample area of Hare Fiord, where the fiord water reached depths of only 3–4m, these same species are found in relative abundance at 8 m, the shallowest sample depth.

It is more difficult to explain the apparent limitation of certain species to the depths affected by fiord water. Examples of this distribution pattern are illustrated by *Nereimyra punctata*, *Pectinaria granulata*, and *Sabellides borealis*, which were collected almost exclusively in depths of 8 m and less. The distributions of *Euchone papillosa* and *Pholoe minuta* also seem to indicate a marked preference for the depths affected by the fiord water, although they are found in reduced numbers at greater depths.

All species living at these shallow depths are capable of existing under the hydrographic conditions of deeper areas in the fiord and, indeed, they do live under these conditions whenever the fiord water does not penetrate very deeply. The somewhat coarser sediment at depths less than 10 m could account for the unusually high population densities of these species. It is also possible that the presence of fiord water excludes other benthic species that act as predators or competitors under normal conditions.

## Acknowledgments

I gratefully acknowledge the guidance of Dr M. J. Dunbar, Marine Sciences Centre, McGill University, during the M.Sc. thesis project on which this paper is based (Curtis 1970). Dr J. W. Wacasey, Arctic Biological Station, Fisheries Research Board of Canada, patiently instructed me in polychaete taxonomy and critically reviewed the manuscript. Logistics for field work were provided by Operation Tanquary (Defence Research Board of Canada), directed by Dr G. Hattersley-Smith. I received the help of Mr. Harold Serson (Defence Research Board) on many technical matters. I wish to thank Mr D. Farmer for his assistance in the field and for preparing computer programs used in quantitative analysis.

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Cairns, A. A. 1967. The zooplankton of Tanquary Fiord, Ellesmere Island, with special reference to the calanoid copepods. J. Fish. Res. Bd. Canada 24: 555–568.

Clark, R. B., and A. Milne. 1955. The sublittoral fauna of two sandy bays on the Isle of Cumbrae, Firth of Clyde. J. Mar. Biol. Ass. U.K. 34: 161–180.

Curtis, M. A. MS 1970. Depth distributions of benthic polychaetes in Hare Fiord and Tanquary Fiord, Ellesmere Island, N.W.T. McGill Univ. Marine Sciences Centre MS Rep. 16: 62 p.

ELLIS, D. V. 1960. Marine infaunal benthos in arctic North America. Arctic Inst. N. Amer. Tech. Pap. 5: 53 p.

FAUVEL, P. 1923. Polychetes errantes p. 1–448. *In* P. Grassé [ed.] Faune de France. Vol. 5. Librarie de la Faculte des Sciences, Paris

FORD, W. L., AND G. HATTERSLEY-SMITH. 1965. On the oceanography of the Nansen Sound fiord system. Arctic 18: 158–171.

MCINTYRE, A. D., AND A. ELEFTHERIOU. 1968. The bottom fauna of a flatfish nursery ground. J. Mar. Biol. Ass. U.K. 48: 113–142.

MILLS, E. L. 1969. The community concept in marine zoology, with comments on continua and instability in some marine communities: a review. J. Fish. Res. Bd. Canada 26: 1415–1428.

PETERSEN, C. G. J. 1913. Valuation of the sea. II. The animal communities of the sea bottom and their importance for marine zoogeography. Rep. Danske Biol. Sta. 21: 44 p.

PETTIBONE, M. H. 1954. Marine polychaete worms from Point Barrow, Alaska, with additional records from the North Atlantic and North Pacific. Proc. U.S. Nat. Mus. 103: 203–256.

1956. Marine polychaete worms from Labrador. Proc. U.S. Nat. Mus. 105: 531–584.

1963. Marine polychaete worms of the New England region. 1. Aphroditidae through Trochochaetidae. U.S. Nat. Mus. Bull. 227: 356 p.

SANDERS, H. L. 1968. Marine benthic diversity, a comparative study. Amer. Natur. 102: 243–282.

SPÄRCK, R. 1933. Contributions to the animal ecology of the Franz Joseph Fjord and adjacent East Greenland waters. Medd. Grønland 100(1): 36 p.

THORSON, G. 1934. Contributions to the animal ecology of the Scoresby Sound fiord complex (East Greenland). Medd. Grønland 100(3): 68 p.

1957. Bottom communities, p. 461–534. *In* J. Hedgepeth, Treatise on marine ecology and paleoecology. Vol. 1. Memoir 67. Geol. Soc. America, New York, N.Y.

USCHAKOV, P. V. 1955. Polychaeta of the Far Eastern Seas of the U.S.S.R. Acad. Nauk U.S.S.R. 56: 445 p. (Transl. from Russian by Israel Program for Sci. Transl., Jerusalem, 1965)

WHITTAKER, R. H. 1967. Gradient analysis of vegetation. Biol. Rev. (Cambridge) 42: 207–264.

# Change in Thermal Regime as a Cause of Reduction of Benthic Fauna Downstream of a Reservoir

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LEHMKUHL, D. M. 1972. Change in thermal regime as a cause of reduction of benthic fauna downstream of a reservoir. J. Fish. Res. Bd. Canada 29: 1329–1332.

The kinds and numbers of Ephemeroptera and other insects in the Saskatchewan River are greatly reduced downstream of a dam. This is attributed to changes in river temperatures caused by the reservoir. The river is warmed in winter and cooled in summer. Consequently, mayflies and other insects with strict thermal requirements cannot hatch and grow successfully. The effect is evident 70 miles downstream.

LEHMKUHL, D. M. 1972. Change in thermal regime as a cause of reduction of benthic fauna downstream of a reservoir. J. Fish. Res. Bd. Canada 29: 1329–1332.

Le nombre d'espèces et d'individus d'éphéméroptères et autres insectes est grandement réduit en aval d'un barrage dans la rivière Saskatchewan. Nous attribuons ce phénomène aux changements de température de la rivière, réchauffement en hiver et refroidissement en été, causés par le bassin de retenue. L'éclosion et la croissance des éphémères et autres insectes sténothermes en sont affectées. Les effets se font sentir à 70 milles en aval du barrage.

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RECENT investigations have shown striking reductions in invertebrates downstream from the outlets of reservoirs (Pearson et al. 1968; Lehmkuhl 1970; Hilsenhoff 1971; Spence and Hynes 1971a, b). The cause of the reduction has not been demonstrated, but lowered water temperature, altered water chemistry, and increase in siltation have been implicated. The objective of this study was to determine the cause of the reduction of fauna, and this report provides evidence that an alteration of the total seasonal temperature regime prevents reproduction of many benthic organisms.

A reservoir results when any watercourse is dammed. In North America, virtually no large temperate river remains that is not regulated to some degree. The Missouri and Tennessee are examples of rivers that have been brought entirely under control by reservoirs (Neel 1963), and other large programs are in the planning stages (Anon. 1969). When a dam is constructed, the upstream riverine environment changes into a lake-like reservoir with associated alteration of the fauna. Changes also occur on the downstream side, because as water flows into the reservoir the sediment load is lost, dissolved materials may be gained or lost, and thermal stratification usually occurs. Reservoirs in temperate climates normally discharge from the bottom (in contrast to natural lakes) a summer cool but winter warm, chemically altered, sediment-free outflow into the downstream channel at a controlled rate. Downstream biological changes (compared to the original river or the river upstream, not the reservoir itself) have been predicted (Edmunds and Musser 1960; Edmunds 1972) and have now been documented (see above), but the mechanism causing the reduction had not been demonstrated.

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## Effects on Study Area

In the present study I have found a marked reduction of macro-invertebrates downstream from a reservoir on the South Saskatchewan River (Fig. 1), and the reduction is evident 70 miles downstream from the dam, at which point sewage and industrial pollution from Saskatoon mask the effect of the reservoir and prevent further downstream monitoring. The conclusion that there was a reduction in the fauna 70 miles downstream from the outlet is based on the following observations. During 1970 and 1971, extensive collections were made in the Saskatchewan River; 19 species of mayflies (Ephemeroptera) were found in the control area upstream from the reservoir (Fig. 1, Station I), and 20 species were collected at or near the control station on the North Saskatchewan River (Fig. 1, Station VII). In contrast, no species were collected below the dam outlet (Station II), one species was collected at Station III, two species were collected at Station IV, three species were collected at Station V, and seven

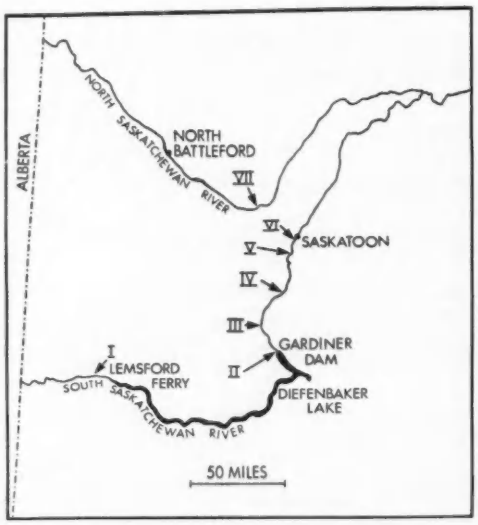


FIG. 1. Study area in west central Saskatchewan. Stations I and VII provide a base for comparison with stations II–VI, which are influenced by the reservoir.

species were collected at Station VI, 70 miles downstream from the outlet. Thus, even 70 miles downstream the incidence of mayfly species was less than 40% compared to the upstream control area and the control area in the north branch of the river.

Invertebrates other than mayflies were also reduced downstream near the outlet. In 1971, the uniform sampling technique used at stations II-VI was a modification of the kick sample method (Spence and Hynes 1971a) and involved sweeping with a long-handled net while one stirs the bottom with the feet and walks backward against the water current. The dislodged organisms are captured in the net. The sample at each station consisted of two 20-ft samples near the shore and one 50-ft sample in a sand bar. The results were as follows: nine chironomid larvae (Diptera) and no other invertebrates were found below the outlet (Station II); stations III–VI, respectively, yielded 39, 42, 277, and 289 specimens of aquatic invertebrates; in terms of diversity, the number of major groups represented (Chironomidae, other Diptera, Corixidae, Dytiscidae, Gomphidae, Plecoptera, Trichoptera, Ephemeroptera, leeches, molluscs, and Crustaceans) were, respectively, 1, 6, 6, 7, and 11 for stations II–VI. The data support the earlier mentioned reports that there is an alteration of the benthic fauna near the outlet of the reservoir, and, further, that the effect extends a number of miles (in this study, 70) downstream from the outlet (also noted by Pearson et al. 1968).

## POSSIBLE ENVIRONMENTAL FACTORS

What factor or factors cause the reduction of the fauna? Since an increase in the density and diversity of the fauna was observed with increasing distance downstream from the outlet (from 0 to 7 species of mayflies, from 1 to 11 groups of invertebrates, and from 9 to 289 total specimens per sample), it is argued that the causal factor should change with the recovery of the fauna. It should be possible to measure environmental factors at points downstream from the outlet, eliminate as insignificant those factors that remain constant at all stations, and retain as significant those factors that change (become more favorable) with the increase in the faunal community. Measurements were taken on total dissolved solids, *p*H, total alkalinity, P alkalinity, Ca hardness, Mg hardness, sulfide, chloride, nitrate, $CO_2$, phosphate, silica, ammonia, turbidity, oxygen, and temperature. All physical and chemical factors measured except temperature were nearly constant at the stations downstream from the reservoir. Organic matter and plant growths for the base of the food chain were available at all stations. The water was saturated with $O_2$ at all stations at midday in summer, and because of the turbulence of the outflow and absence of unusual BOD, there is no reason to believe that $O_2$ levels would be low in other seasons. Total dissolved solids were always near 265 ppm and there were no significant differences in dissolved materials among the stations. Water color and turbidity increased downstream with the increase in organisms but this is probably a result of increased algal growth and agricultural run-off, and not a cause of faunal depletion. Differences among stations were slight and the published literature provided no evidence that minor variations of these factors can cause broad elimination in all groups from a habitat. Temperature, however, does appear to be an important factor.

## EFFECT ON WATER TEMPERATURE

Temperature was obviously altered by the reservoir (Fig. 2). No upper lethal temperatures were involved, so temperature per se at a given time must not be the limiting factor. When the total seasonal temperature pattern is considered and correlated with the life cycles of organisms, it will be shown that it is impossible for certain organisms to reproduce near the dam outlet.

Figure 2 shows the seasonal pattern of warming and cooling at points downstream from the dam

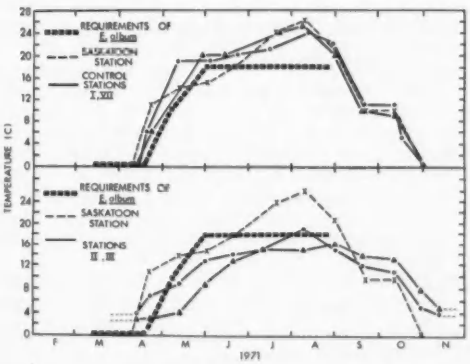


FIG. 2. Seasonal temperature pattern at the control stations (upper) and at stations downstream from Diefenbaker Lake (lower). The pattern for Saskatoon (VI) is repeated to facilitate comparison. Temperatures are the daily maximum — i.e. readings were taken in the afternoon during clear weather; the heavy line summarizes the requirements of *Ephoron album*; exposure of eggs to freezing; an increase to 10–13 C, a further increase to 18 C; 2½ months at or above 18 C.

and the control stations (I and VII, Fig. 1). At stations I and VII (the controls) the temperature increased rapidly in April after ice break-up and dropped sharply in the autumn, leading to freezing of the river. The temperature was 0 C (ice) from November until April. In contrast, within 20 miles of the outlet (stations II and III, Fig. 1) the water temperatures did not drop sufficiently to form an ice cover and the spring rise and autumn decline in temperature were gradual. Summer maxima were lower and winter minima were higher at stations II and III than at I and VII.

## EFFECTS ON MAYFLIES

The life cycles of most of the mayflies (Ephemeroptera) of the area were determined, and it is possible to correlate these life cycles with the temperature patterns. Ten species of mayflies from the study area (*Isonychia sicca* (Walsh), *Lachlania saskatchewanensis* Ide, *Anepeorus rusticus* McDunnough, *Epeorus* sp., *Choroterpes albiannulata* McDunnough, *Traverella albertana* (McDunnough), *Ephemerella* sp., *Brachycercus prudens* (McDunnough), and *Ephoron album* (Say.) *Pseudiron centralis?* McDunnough) fall clearly into Hynes' (1970) *fast seasonal* cycle, in which the eggs have a long period of diapause (arrested development) and hatch after the appropriate environmental stimuli. Following hatching there is rapid growth of the nymphs leading to emergence of adults. The above-listed mayflies pass the winter in the egg stage; depending on the species, the nymphs hatch in June, July, or August and the adults appear in August or September of the same year. Experimental results are available for *E. album* (Britt 1962). With reference to temperature the species has three requirements: the eggs will not complete their development unless they are exposed to a period of temperature at or near freezing (breaking of diapause) (Britt 1962, found that 1.3 C was not sufficiently low to allow normal development); second, the eggs will not hatch to nymphs unless the near-zero temperature is followed by temperatures of 10–13 C; third, the nymphs will not reach maturity unless exposed to 2½–4 months of warm temperatures (not specified by Britt, but apparently 18–28 C). As can be seen in Fig. 2, all these requirements were met in the control areas where all the above species of the *fast seasonal* type were present, but the requirements for both freezing and warmth were not present near the dam where the above species were absent. Even if the freezing requirement was met, the requirements for hatching and growth were not. I conclude that the species are eliminated by an altered water temperature pattern.

Five species of mayflies (*Ephemera simulans* Walker, *Ametropus neavei* McDunnough, *Siphloplecton* sp., *Baetisca bajkovi* Neave, and a species belonging to an undescribed genus) fall into Hynes' (1970) *slow seasonal* cycle, wherein the eggs hatch soon after they are laid (no diapause) and maturation of the immature stages requires approximately 1 year. In the case of the above species at Station I, eggs were laid and hatched in summer, much growth occurred during the warm period of late summer and autumn, and nymphs wintered under the ice, at which time growth was limited, and adults occurred the following summer. It has been observed that *Ephemera simulans* Walker requires a specific number of degree-days (sum of mean daily temperatures) in spring before the nymphs emerge to adults (Britt 1962). At the control stations I and VII, warm periods were present for the development of these species (Fig. 2), and the species were present. In areas near the dam the warm periods were much curtailed (Fig. 2), and the species under consideration were absent. I conclude that these species are also eliminated by the altered temperature pattern.

## Discussion

I conclude that the 15 species listed above cannot successfully reproduce and thus are absent near the dam outlet for one or both of two reasons: the required temperature sequence for breaking

diapause and stimulating egg hatching and growth is absent, or the total degree-days (especially in spring) needed for growth and adult emergence are not attained. Solid data are presented for two species (*Ephoron album* and *Ephemera simulans*), and inferences based on the presented data are made for 13 additional mayfly species. This same argument apparently applies to a wide range of organisms. Hynes (1970) shows that aquatic invertebrates other than mayflies, in fact most insects, have *fast* and *slow* seasonal life cycles, and would have similar requirements.

Consequently, I make the inference that the non-mayflies were eliminated by the same process as were the mayflies. Finally, I infer that all deep reservoirs in temperate climates (i.e. reservoirs that stratify thermally) that have a hypolimnion drain will cause similar downstream faunal depletion, and this explains the faunal depletion in other areas of North America.

## Acknowledgments

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ANON. 1969. Saskatchewan-Nelson Basin Board annual report. Dep. Energy Mines Resour. Ottawa, Ont., Canada.

BRITT, N. W. 1962. Biology of two species of Lake Erie mayflies, *Ephoron album* (Say) and *Ephemera simulans* Walker. Bull. Ohio Biol. Surv. 5: 70.

EDMUNDS, G. F., JR. 1972. Trends and priorities in mayfly research. First Int. Conf. Ephem., Tallahassee, Florida. (In press)

EDMUNDS, G. F., JR., AND G. G. MUSSER. 1960. The mayfly fauna of Green River in the Flaming Gorge Reservoir Basin, Wyoming and Utah, p. 111–123. *In* A. M. Woodbury [ed.] Ecological studies of the flora and fauna of Flaming Gorge Reservoir Basin, Utah and Wyoming. Anthropological Papers, 48. Univ. of Utah Press, Salt Lake City, Utah.

HILSENHOFF, W. L. 1971. Changes in the downstream insect and amphipod fauna caused by an impoundment with a hypolimnion drain. Ann. Entomol. Soc. Amer. 64: 743–746.

HYNES, H. B. N. 1970. The ecology of running waters. Univ. of Toronto Press, Toronto, Ont. 555 p.

LEHMKUHL, D. M. 1970. Mayflies in the South Saskatchewan River; pollution indicators. Sask. Nat. Hist. Soc. 28: 183–186.

NEEL, J. K. 1963. Impact of Reservoirs, p. 575–593. *In* C. G. Frey [ed.] Limnology in North America, Univ. Wisconsin Press, Madison, Wis.

PEARSON, W. D., R. H. KRAMER, AND D. R. FRANKLIN. 1968. Macroinvertebrates in the Green River below Flaming Gorge Dam, 1964–65 and 1967. Proc. Utah Acad. Sci. Arts Lett. 45: 148–167.

SPENCE, J. A., AND H. B. N. HYNES. 1971a. Differences in benthos upstream and downstream of an impoundment. J. Fish. Res. Bd. Canada 28: 35–43.

1971b. Differences in fish populations upstream and downstream of a mainstream impoundment. J. Fish. Res. Bd. Canada 28: 45–46.

# Effects of Exposure to Sublethal DDT on the Locomotor Behavior of the Goldfish (*Carassius auratus*)

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DAVY, F. B., H. KLEEREKOPER, AND P. GENSLER. 1972. Effects of exposure to sublethal DDT on the locomotor behavior of the goldfish (*Carassius auratus*). J. Fish. Res. Bd. Canada 29: 1333–1336.

A highly significant time-dependent correlation between consecutive turns in the locomotor pattern of normal goldfish (*Carassius auratus*), attributed to a "memory" process in the pertaining locomotor control mechanism in the central nervous system, is significantly reduced within 4 days by chronic exposure of the fish to 10 μg/liter p.p′. DDT. Keeping the fish in clean water during 130–139 days did not result in the restoration of the above correlation.

DAVY, F. B., H. KLEEREKOPER, AND P. GENSLER. 1972. Effects of exposure to sublethal DDT on the locomotor behavior of the goldfish (*Carrassius auratus*). J. Fish. Res. Bd. Canada 29: 1333–1336.

La corrélation très significative, liée au temps, entre les virages consécutifs faits au cours de la locomotion chez des individus normaux de *Carassius auratus* et attribuables à un processus de "mémoire" du centre locomoteur approprié du système nerveux central est fortement réduite, en moins de quatre jours, chez des animaux exposés à une concentration de 10 μg/litre de *pp′* DDT. Le retour des poissons à un milieu d'eau pure ne rétablit pas la corrélation, même après une période de 130 à 139 jours.

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EXTENSIVE information is available on the susceptibility of fish to chlorinated hydrocarbons, particularly with respect to the acute toxicity of these pesticides as affected by levels of concentration, exposure time, and a variety of experimental conditions. Although as early as the 1940s evidence began to accumulate that pointed to damaging effects of DDT on neural elements and the central nervous system (Aubin and Johansen 1969; Kayser et al. 1962; Welsh and Gordon 1947; Dale et al. 1963) and neural functions in a variety of animals, including fish (Desi et al. 1966; O'Brien and Matsumura 1964; Revzin 1966; Aubin and Johansen 1969), only recently have inquiries been initiated into the effects of chronic exposure to sublethal concentrations of DDT on sensory and behavioral processes (Anderson 1968; Ogilvie and Anderson 1965; Javaid MS 1967; Saunders 1969; Johansen MS 1963; Warner et al. 1966; Anderson and Prins 1970; Jackson et al. 1970).

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In the course of an extensive analysis of the locomotor behavior of the goldfish (*Carassius auratus*) a significant correlation was established between the magnitudes of consecutive turns in opposite directions, which initially decreases gradually as the time between the turns increases and then creases abruptly (Westlake and Kleerekoper 1970). Since a "memory" or retention process was involved in the behavior described, it may be assumed that at least that aspect of turning behavior is controlled by the central nervous system. The effects of chronic exposure to a sublethal dose of DDT on the above phenomenon are reported in this paper.

## Materials and Methods

### MONOTOR TANK

The technique for the monitoring of locomotion and the processing of the data used in this study were described earlier (Kleerekoper 1969; Kleerekoper et al. 1970). The monitor tank was supplied with water, which was not recirculated, from the same source as that used for

TABLE 1. Characteristics of laboratory water. (Values in *milligrams per liter* as indicated.)

| | |
|---|---|
| Carbonates ($-CO_3$) | 10 |
| Bicarbonates ($-HCO_3$) | 152 |
| Chlorides | 47.5 |
| Sulphates ($-SO_4$) | 10.7 |
| Silica ($SiO_2$) | 18.5 |
| $Ca^{++}$ | 1.8 |
| $Mg^{++}$ | 0.2 |
| $Na^{+}$ | 99.4 |
| Hardness ($CaCO_3$) | 5.4 |
| $Cu^{++}$ | 0.006 |
| $Zn^{++}$ | 0.002 |
| Conductivity $\mu$mho | 162 |
| *p*H | 8.4 |

the DDT treatment. Flow across the tank was maintained at a rate of 1.8 cm/min with a mean water temperature of 26.2 C.

### TREATMENT OF FISH

Goldfish 19–31 cm total length were obtained from a commercial grower in Texas. All fish were kept individually in fiberglass tanks (56 × 48 × 33 cm deep) in 100 liters of stagnant, aerated water maintained at a mean temperature of 21 C. Water used in both the holding tanks and the monitor tank first passed through an activated carbon filter and had the chemical characteristics listed in Table 1. All fish were fed the same dry balanced fish food and had their water changed once every 24 hr while in the holding tanks.

The locomotor behavior of all 19 fish used in these experiments was monitored for 24 hr prior to experimental treatment. Seven of these fish were exposed to DDT (referred to as "DDT-treated") for 4 days, by the following procedure. Once every 24 hr each fish was transferred to a clean tank containing 100 liters of water to which a solution of *p.p'* DDT (Geigy, 99.9%) in 4 ml of acetone was added to produce a pesticide concentration of 10 $\mu$g/liter of water. The fish were transferred every 24 hr because of the well-known difficulties of maintaining a constant concentration of DDT due to its low solubility in water and its ready adhesion to surfaces (Holden 1962; Biggar et al. 1967).

Another four fish (referred to as "acetone-treated") were treated similarly but DDT was not added to the acetone. The remaining eight fish (referred to as "controls") were subjected to the above treatment but for the addition of the DDT–acetone mixture. At the end of the 4-day treatments the locomotor behavior of individual fish was again monitored for 24 hr. At no time has DDT been used in the monitor tank.

### DATA TREATMENT

The data consisted of various values of correlation coefficients, between the magnitudes of consecutive turns, in each of the 20, 0.5-sec time intervals. Due to the varying number of data in each of these time intervals, the following grouping procedure was followed to insure that all tests were conducted with similar statistical power. If any correlation coefficient was insignificant, it was then pooled with the correlation coefficient of the following time interval. This procedure was repeated until a significant correlation coefficient was obtained (95% confidence limit) (Snedecor and Cochran 1968).

The data from the three conditions (DDT-treated, acetone-treated, and control) were subjected to a two-way analysis of variance according to the model: $x_{ij} = \mu + F_i + D_j + FD_{ij}$, where the $F_i$ represented a fish effect ($i$ = 1–19), $D_j$ represented a day effect ($j$ = 1 ,2) and $FD_{ij}$ represented the possible fish day interaction. The fish effect was further analyzed by a set of contrasts, testing the mean of the DDT fish versus the mean of the control fish, and the mean of the acetone fish versus the mean of the DDT fish.

## Results

The results of pooling successive 0.5-sec intervals for a representative experiment are shown in Fig. 1. The time at which the correlation coefficient remained insignificant for all 19 fish is shown in Table 2. A reduction in this time is seen in the DDT-treated fish by day 4. The DDT effect as shown by the analysis of variance was highly significantly different ($\alpha$ = .01) from both the untreated fish and those subjected to acetone treatment alone. Therefore, these data indicate that the reduction in time at which the correlation between the magnitudes of consecutive turns is maintainted resulted from DDT treatment rather than daily transfer or exposure to acetone. The permanence of this effect was studied in two of the seven experimental fish. Here the correlation was not restored by discontinuation of the DDT treatment at 16 days and their subsequent

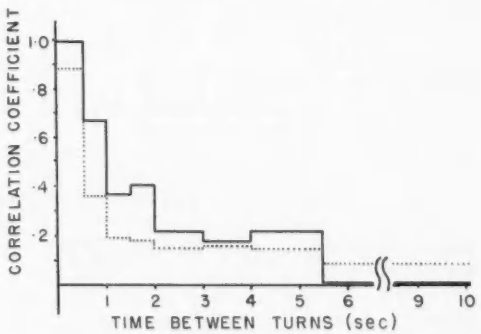


FIG. 1. Correlation coefficients between consecutive turns for grouped data; dotted line indicates the 95% confidence limit. (Note: differing values for the 95% confidence limit are due to the varying number of turns in each time category.)

TABLE 2. Interval of time (*sec*) between the magnitudes of consecutive turns during which the correlation coefficient remains significant (95% confidence limit).

| DDT treated | | | Control | | | Acetone treated | | |
|---|---|---|---|---|---|---|---|---|
| | Day | | | Day | | | Day | |
| Fish no. | 0 | 4 | Fish no. | 0 | 4 | Fish no. | 0 | 4 |
| 1 | 6.5 | 1.5 | 8 | 5.5 | 4.0 | 16 | 3.5 | 6.5 |
| 2 | 9.5 | 0.0 | 9 | 4.5 | 4.0 | 17 | 10.0 | 4.5 |
| 3 | 7.5 | 6.0 | 10 | 10.0 | 9.5 | 18 | 6.0 | 6.0 |
| 4 | 7.0 | 1.5 | 11 | 9.5 | 7.5 | 19 | 7.5 | 8.0 |
| 5 | 8.0 | 3.0 | 12 | 10.0 | 7.0 | | | |
| 6 | 7.0 | 4.0 | 13 | 9.5 | 7.0 | | | |
| 7 | 4.0 | 3.0 | 14 | 7.0 | 4.5 | | | |
| | | | 15 | 7.5 | 9.0 | | | |

maintenance in clean water during 130 and 139 days.

Pesticide residue analysis was carried out by gas–liquid chromatography, using methods from the FDA pesticide analytical manual. Results for four fish exposed to 10 µg/liter DDT for 4 days experimental and three untreated controls were as follows:

| | DDT (*ppm*) | DDE (*ppm*) | TDE (*ppm*) |
|---|---|---|---|
| Control (136–199 *g*) | 0.018–0.025 | 0.024–0.030 | 0.006–0.011 |
| Experimental (146–221 *g*) | 0.90–1.11 | 0.54–0.62 | 0.051–0.11 |

## Discussion

Successive turns made by normal goldfish in the course of unrestricted spontaneous locomotion display a highly significant time-dependent correlation of their magnitudes. This correlation decreases as the elapsed time between the turns increases and it becomes insignificant at time values in excess of a few seconds. The phenomenon indicates the presence of a short-term retention or "memory" trace following the execution of a turn and affecting the performance of the next turn (Westlake and Kleerekoper 1970). The results here presented demonstrate that the above correlation between turns is significantly affected by exposing the fish to water containing DDT at 10 µg/liter when prepared and renewed daily during 4 days. The correlation becomes limited to a much shorter elapsed time value, i.e. the retention is significantly reduced. It may be postulated, therefore, that DDT impaired the retention mechanism involved in the correlation of consecutive turns and, therefore, in the establishment of the fish locomotor pattern (Kleerekoper et al. 1970). In this connection, some of the results obtained by Anderson and Peterson (1969) are of particular interest. In the course of an investigation on the effects of DDT on the cold-blocking temperature of the tail reflex in brook trout, those authors provided evidence that exposure to DDT at 20 ppb for 24 hr may have converted previously trained fish into naive ones but for some residue of conditioning from training. Although this was not stated by the authors, the results imply that the retention of what had been learned may have been destroyed, at least in part. However, Jackson et al. (1970), using a conditioned avoidance response in Atlantic salmon and speckled trout, found no effect of prior DDT treatment at 20 ppb for 24 hr on the ability of the fish to acquire the conditioned response and retain it for 24 hr.

The impairment described here was obtained at a lower concentration of DDT than those that produced definable behavioral effects in fish reported by other workers. At the time when the loss of correlation between turns was measured, the whole fish had accumulated DDT, DDE, and TDE (as shown above) at concentrations well within values reported for fish in the natural environment. For example, a survey of 28 freshwater species from the Great Lakes yielded average concentrations between 0.25 (spottail shiner, *Notropis hudsonius*, Lake Erie) and 7.44 ppm DDT (DDT, DDE, DDD) (lake trout, *Salvelinus namaymsh*, Lake Superior), with goldfish at 0.70 ppm (Reinert 1970). Other surveys have produced comparable values, although individual variability within a population is usually high.

The long-lasting effect of the DDT treatments, tested in only two goldfish, is in disagreement with the results obtained by Anderson and Prins (1970), who reported an adverse effect of DDT on learning in brook trout, which disappeared within 3 to 4

weeks after the treatment was stopped. However, the fish in these experiments had been exposed to 25 ppb for 24 hr prior to testing, whereas the results here reported refer to 16 days of exposure at 10 ppb, followed by 130 and 139 days in laboratory water without DDT.

Effects of DDT exposure on locomotor behavior have not been described in any detail in the literature. Anderson and Peterson (1969) found no impairment of gross swimming behavior in brook trout, but Johansen (1963) reported that DDT-treated Atlantic salmon showed a uniformity of locomotor activity not observed in the controls and that the effect of temperature on maximum activity also differed in the two groups. No additional information is available on these aspects of locomotion

## Acknowledgments

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ANDERSON, J. M. 1968. Effect of sublethal DDT on the lateral line of brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Canada 25: 2677–2682.

ANDERSON, J. M., AND M. R. PETERSON. 1969. DDT: sublethal effects on nervous system function in brook trout (*Salvelinus fontinalis*). Science 164: 440–441.

ANDERSON, J. M., AND H. B. PRINS. 1970. Effects of sublethal DDT on a simple reflex in brook trout. J. Fish. Res. Bd. Canada 27: 331–334.

AUBIN, A. E., AND P. H. JOHANSEN. 1969. The effects of an acute DDT exposure on the spontaneous electrical activity of goldfish cerebellum. Can. J. Zool. 47: 163–166.

BIGGAR, J. W., G. R. DUTT, AND R. L. RIGGS. 1967. Predicting and measuring the solubility of *p, p'* DDT in water. Bull. Environ. Contam. Toxicol. 2: 90–100.

DALE, W. E., T. B. GAINES, W. J. HAYES, AND G. W. PEARCE. 1963. Poisoning by DDT: relation between clinical signs and concentration in rat brain. Science 142: 1474.

DESI, I., I. FARKAS, AND T. KEMENY. 1966. Changes of central nervous function in response to DDT administration. Acta Physiol. Sci. Hung. Tomus 30: 275–282.

HOLDEN, A. V. 1962. A study of the absorption of $^{14}C$-labelled DDT from water by fish. Ann. Appl. Biol. 50: 467–477.

JACKSON, D. A., J. M. ANDERSON, AND D. R. GARDNER. 1970. Further investigations of the effect of DDT on learning in fish. Can. J. Zool. 48: 577–580.

JAVAID, M. Y. MS 1967. Some factors affecting the temperature selection of 3 species of salmonids. M.S. Thesis. Carleton Univ., Ottawa, Ont.

JOHANSEN, P. H. MS 1963. The effects of sublethal concentrations of DDT on locomotor activity and rate of growth of Atlantic salmon parr, *Salmo salar*, L. M.S. Thesis. Univ. of New Brunswick, Fredericton, N.B.

KAYSER, H., A. LUEDEMANN, AND H. NEUMANN. 1962. Nerve cell injuries caused by insecticides poisoning in fishes and land crabs. Z. Angew. Zool. 49: 135–48.

KLEEREKOPER, H. 1969. Olfaction in fishes. Indiana University Press, Bloomington and London. 222 p.

KLEEREKOPER, H., A. M. TIMMS, G. F. WESTLAKE F. B. DAVY, T. MALAR, AND V. M. ANDERSON. 1970. An analysis of locomotor behaviour of the goldfish. Anim. Behav. 18: 317–330.

O'BRIEN, R. D., AND F. MATSUMURA. 1964. DDT: a new hypothesis of its mode of action. Science 146: 657–658.

OGILVIE, D. M., AND J. M. ANDERSON. 1965. Effects of DDT on temperature selection by young Altlantic salmon, *Salmo salar*. J. Fish Res. Bd. Canada 22: 503–512.

REINERT, R. E. 1970. Pesticide concentrations in Great Lakes fish. U.S. Dep. Health Educ. Welf. 3: 223–240.

REVZIN, A. M. 1966. Effects of endrin on telencephalic function in the pigeon. Toxicol. Appl. Pharmacol. 9: 75–83.

SAUNDERS, J. W. 1969. Mass mortalities and behavior of brook trout and juvenile Atlantic salmon in a stream polluted by agricultural pesticides. J. Fish. Res. Bd. Canada 26: 695–699.

SNEDECOR, G. W., AND W. G. COCHRAN. 1968. Statistical methods. 6th ed. Iowa State University Press, Ames, Iowa. 593 p.

WARNER, R. E., K. K. PETERSON, AND L. BORGMAN. 1966. Behavioural pathology in fish: a quantitative study of sublethal pesticide toxication. *In* Pesticides in the environment and their effects on wildlife. J. Appl. Ecol. (Suppl.) 3: 223–247.

WELSH, J. H., AND H. T. GORDON. 1947. The mode of action of certain insecticides on the arthropod nerve axon. J. Cell. Comp. Physiol. 30: 147.

WESTLAKE, G. F., AND H. KLEEREKOPER. 1970. Evidence for a memory process in the turning behavior of free swimming goldfish. Can. J. Zool. 48: 813–815.

# NOTES

## Biology of *Gammarellus angulosus* (Crustacea, Amphipoda) in the Northwestern Atlantic[1,2]

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Steele, D. H., and V. J. Steele. 1972. Biology of *Gammarellus angulosus* (Crustacea, Amphipoda) in the northwestern Atlantic. J. Fish. Res. Bd. Canada 29: 1337–1340.

*Gammarellus angulosus* is a littoral Atlantic amphipod found from eastern Newfoundland south to the Cape Cod region. It is found clinging to algae of highly exposed rocky shores. The life cycle is 1 year. Fifty percent maturity of the females occurs at 8.8 mm. Mature females produce successive broods, except between September and November when they are in the resting stage. Fecundity increases with the size of the female. Young of the November brood are released from the females in May and adults disappear in the summer.

Steele, D. H., and V. J. Steele. 1972. Biology of *Gammarellus angulosus* (Crustacea, Amphipoda) in the northwestern Atlantic. J. Fish. Res. Bd. Canada 29: 1337–1340.

*Gammarellus angulosus* est un amphipode dispersé sur le littoral atlantique, depuis l'est de Terre-Neuve jusqu'à la région du cap Cod au sud. On le trouve accroché aux algues des plages rocheuses fortement exposées. La durée de son cycle vital est d'un an. La longueur à laquelle 50% des femelles sont matures est de 8.8 mm. Les femelles matures produisent des portées successives, sauf de septembre à novembre, alors qu'elles sont au stade de repos. La fécondité augmente en proportion de la taille de la femelle. Les jeunes de la portée de novembre sont libérés en mai, et les adultes disparaissent en été.

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This paper presents data on some aspects of the biology of *Gammarellus angulosus* in the northwestern Atlantic, as represented by collections made at Logy Bay, Nfld. It is a part of a larger study of intertidal and shallow water macrocrustacea. The biological and ecological significance of these data and observations will be apparent upon comparison with related publications (e.g. Steele and Steele 1969, 1970a) and will be discussed at length in a forthcoming publication.

The present species has not been studied previously and its validity has been questioned by many authors, such as Stephensen (1940) who considered it to be a synonym of *Gammarellus homari*. However, a comparison of its morphology, ecology, and biology with *G. homari* (Steele 1972) shows it to differ in many respects and it is undoubtedly a distinct species as has been concluded by Bousfield (1956) and others.

In the northwestern Atlantic, *Gammarellus angulosus* has been found from northeastern Newfoundland (author coll.) and Cape Gaspé (Bousfield 1956) south to the Cape Cod region (Holmes 1904). Its distribution in the eastern Atlantic is somewhat uncertain as often it has not been distinguished from *Gammarellus homari*. Stephensen (1940) summarizes its distribution as extending from the Murman coast south to that of the French Mediterranean.

*Methods* — The Newfoundland population was studied at Logy Bay. Collections were made on the shore of Dyers Gulch below the Marine Sciences Research Laboratory once a month (with a few exceptions) between January 1968 and June 1969 at the time of low water of spring tides. A few earlier collections have also been included. The animals were obtained by sweeping a dip net through the algae, mostly *Alaria*, which occurs abundantly at low water level. They were analyzed in the same way as for *Gammarus duebeni* (Steele and Steele 1969).

The size classes of the oogonia and the sizes of the developing embryos are given below. For convenience all egg stages in the ovary are referred to as oogonia in the text.

[1]Studies in biology from Memorial University of Newfoundland No. 330.

[2]Contribution No. 109 from the Marine Sciences Research Laboratory.

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*Gammarellus angulosus:* size classes of preserved oogonia and oocytes (5% formalin).

| Class | Mean size range, *mm* | Description |
|---|---|---|
| 1 (oogonia) | <0.19 | clear |
| 2 (oocytes) | 0.20–0.29 | white, opaque |
| 3 ( " ) | 0.30–0.39 | yellow, " |
| 4 ( " ) | 0.40–0.49 | yellow, " |
| 5 ( " ) | 0.50> | yellow, " |

*Gammarellus angulosus:* sizes of preserved embryos (5% formalin).

| Stage | No. | Mean size, *mm* | Range | S.E. |
|---|---|---|---|---|
| A | 17 | .650 | .635– .680 | .003 |
| B | 10 | .763 | .750– .780 | .004 |
| C | 10 | .833 | .800– .880 | .009 |
| D | 10 | .888 | .850– .930 | .009 |
| H | 10 | 2.06 | 2.0 –2.10 | .011 |

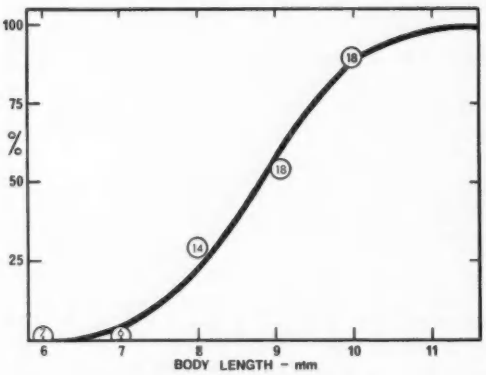


FIG. 1. Size at maturity of female *Gammarellus angulosus* at Logy Bay. Numbers of specimens examined are indicated.

*Environment* — Mean surface temperatures recorded at Logy Bay range from –1.1 C in March to 11.5 C in September (Steele and Steele 1970). At the known southern limit of its distribution the temperatures would be approximated by those from Boston, Mass. where the maximum mean surface temperature in August is 18.8 C (Anon. 1960; Steele and Steele 1970b).

*Gammarellus angulosus* was collected on rocky shores where it was clinging to algae at low water level. It was found to be most abundant on highly exposed shores and occurred only infrequently on those where the exposure is moderate, and not at all on sheltered shores.

The body length at which 50% of the females were mature was calculated to be 8.8 mm (Fig. 1). In the subsequent analysis, only those females 10 mm or longer are considered.

*Size of embryos* — The mean size (length + width/2) of 17 preserved stage A embryos was found to be .650 mm (range .635–.680, S.E. = .003). They increase in size with development (see table).

*Life cycle and reproductive cycle* — Figures 2 and 3 show that mature females may produce a series of broods. When one is developing in the brood pouch, the oogonia of the next enlarge in the ovary (Fig. 3). Most of the adults disappear and presumably die in August–September, but those present in the collections suggest that by September the oogonia fail to enlarge and the females enter the resting stage with the loss of the oostegite setae. This resting stage continues until November when the oogonia begin to enlarge. A few of the broods may be deposited in the brood pouch as early as January, but most not until March. The young may be released from the brood pouch by May, but large numbers were not collected until June or July.

It appears that in this species there is a resting stage between September and November and that several broods of young are released between May and October. The young mature by the following winter or spring. However, individuals do not survive through more than

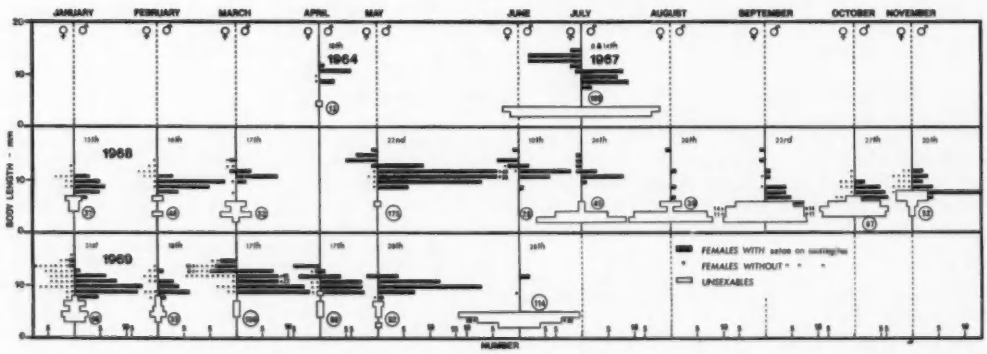


FIG. 2. Monthly size compositions of males and females of *Gammarellus angulosus* collected at Logy Bay. The numbers of specimens examined are shown in circles.

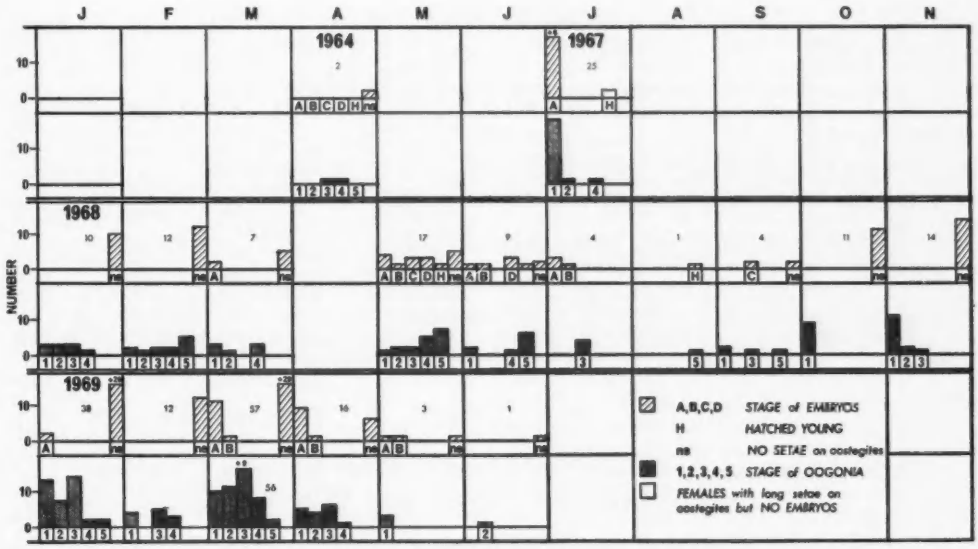


FIG. 3. Frequencies of female *Gammarellus angulosus* with various stages of embryos and oogonia. The number of specimens examined is shown for each where different.

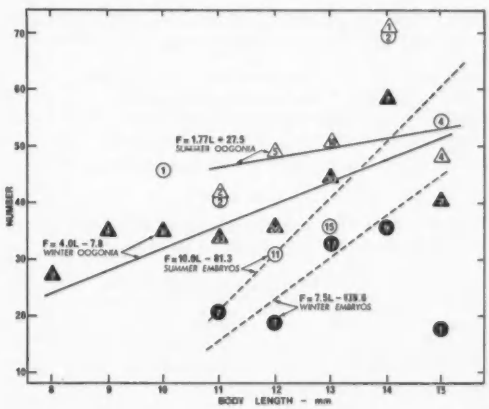


FIG. 4. Numbers of oogonia and embryos per female at Logy Bay. Numbers of specimens examined are inserted inside the symbol that denotes the mean number for a particular size of female.

one reproductive season and the life cycle is essentially annual.

The numbers of enlarged oogonia and embryos were counted for the summer (June–November) and winter (December–May) collections (Fig. 4). The regression lines of the oogonia are lower in the winter period, but this is mainly due to lack of small females in the summer samples. The differences are slight for the larger females.

In order to compare the numbers of oogonia with those of the embryos it is necessary to mentally shift the regression lines of the oogonia about 1 mm to the right. This is to account for the growth of the females when the oogonia are deposited in the brood pouch after fertilization. Although there are considerable differences in the slopes of the regression lines, the comparisons suggest that there is relatively little reduction in number.

*Discussion* — *Gammarellus angulosus* matures at a moderate size and has a moderate fecundity compared to other amphipods of the family Gammaridae. Each female can produce several successive broods but there is a resting period from September to November, so that young are not released during the winter months. This species has an annual life cycle.

This is similar to what has been found in the several *Gammarus* species (*duebeni*, *obtusatus*, and *oceanicus*) which are comparable in size and have similar geographic distributions (Steele and Steele 1969, 1970a, 1972). On the other hand, *Gammarellus carinatus* produces its two broods between December and April in the Black Sea (Greze 1964).

*Acknowledgments* — This study was supported by a grant from the National Research Council of Canada. The assistance of W. O'Brien and C. Long in the analyses of the collections and the preparation of the illustrations is gratefully acknowledged.

ANON. 1960. Water temperature and salinity, Atlantic coast, North and South America. Coast and Geodetic Survey, U.S. Dep. Comm., C. and G.S. Publ. 31–1.

BOUSFIELD, E. L. 1956. Malacostracan crustaceans from the shores of western Nova Scotia. Proc. N. S. Inst. Sci. 24(1): 25–38.

GREZE, I. I. 1964. Zhiznennyi tsikl bokoplava *Gammarellus carinatus* (Rathke) v Tchernom morye. Tr. Sevastopol Biol. Sta. *In* A. D. Kovalenskogo Akad. Nauk SSSR 17: 293–303.

HOLMES, S. J. 1904. The amphipoda of southern New England. Bull. U.S. Bur. Fish. 24: 457–529.

STEELE, D. H. 1972. Some aspects of the biology of *Gammarellus homari* (Crustacea, Amphipoda) in the northwestern Atlantic. J. Fish. Res. Bd. Canada 29: 1340–1343.

STEELE, D. H., AND V. J. STEELE. 1969. The biology of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. I. *Gammarus duebeni* Lillj. Can. J. Zool. 47: 235–244.

1970a. The biology of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. III. *Gammarus obtusatus* Dahl. Can. J. Zool. 48: 989–995.

STEELE, V. J., AND D. H. STEELE. 1970b. The biology of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. II. *Gammarus setosus* Dementieva. Can. J. Zool. 48: 659–671.

1972. The biology of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. V. *Gammarus oceanicus* Segerstråle. Can. J. Zool. (In press)

STEPHENSEN, K. 1940. The Amphipoda of North Norway and Spitsbergen with adjacent waters. Fasc. 3. Tromsø Mus. Skr. 3: 279–362.

# Some Aspects of the Biology of *Gammarellus homari* (Crustacea, Amphipoda) in the Northwestern Atlantic[1,2]

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STEELE, D. H. 1972. Some aspects of the biology of *Gammarellus homari* (Crustacea, Amphipoda) in the northwestern Atlantic. J. Fish. Res. Bd. Canada 29: 1340–1343.

*Gammarellus homari* is a sublittoral Atlantic amphipod found from eastern Newfoundland and the northern Gulf of St. Lawrence north to Ellesmere Island. It is distinguished from *G. angulosus* by its larger size, smaller eyes and when alive by its red coloration. It appears to produce a single brood of young per year. Ovigerous females are found at depths of 12–15 m in the winter and the young are released in late winter or spring. It is not known where the males occur or where the adult females spend the remainder of the year.

STEELE, D. H. 1972. Some aspects of the biology of *Gammarellus homari* (Crustacea, Amphipoda) in the northwestern Atlantic. J. Fish. Res. Bd. Canada 29: 1340–1343.

*Gammarellus homari* est un amphipode sublittoral de l'Atlantique réparti depuis l'est de Terre-Neuve et le nord du golfe Saint-Laurent jusqu'à l'île Ellesmere au nord. Il se distingue de *G. angulosus* par sa taille plus grande, ses yeux plus petits et, vivant, par sa coloration rouge. L'espèce ne semble produire qu'une portée par année. On trouve des femelles ovigères à des profondeurs de 12 à 15 m en hiver, et les jeunes sont libérés à la fin de l'hiver ou au printemps. On ignore où se trouvent les mâles et où les femelles passent le reste de l'année.

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THE present paper summarizes data relating to some aspects of the biology of *Gammarellus homari* for comparison with those of *G. angulosus* (Steele and Steele 1972). It is shown that these are quite different and this study supports the conclusion that *G. homari* is a distinct species.

Since there is no published data on the biology of *G. homari*, it was considered that the results of the present study should be made available, even though only a few specimens could be obtained for examination.

*Materials and methods* — Specimens of *G. homari* were obtained infrequently at LW at Dyers Gulch below the site of the Marine Sciences Research Laboratory

[1]Studies in biology from Memorial University of Newfoundland No. 331.

[2]Contribution No. 110 from the Marine Sciences Research Laboratory.

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at Logy Bay. Moderate numbers of large specimens were discovered by Dr J. M. Green at a depth of 12–15 m below LW in February and March of 1970. They could not be found there in April and in subsequent months. Only several years earlier specimens were obtained in April.

The animals were analyzed in the same manner as for *Gammarus duebeni* (Steele and Steele 1969). The size classes of the oogonia and the sizes of the embryonic stages are given below. Since no stage A embryos were ever seen, the size of class 5 oocytes was determined from the large oocytes (0.995 mm) from a specimen collected in November 1968.

*Gammarellus homari:* size classes of preserved oogonia and oocytes (5% formalin).

| Class | Mean size, *mm* | Description |
|---|---|---|
| 1 (oogonia) | 0.10–0.19 | clear |
| 2 (oocytes) | 0.20–0.39 | |
| 3 ( " ) | 0.40–0.59 | |
| 4 ( " ) | 0.60–0.79 | |
| 5 ( " ) | 0.80– | yellow-brown, opaque |

*Gammarellus homari:* sizes of preserved embryos

| Stage | No. | Mean size, *mm* | Range | S.E. |
|---|---|---|---|---|
| A | none available | | | |
| B | " " | | | |
| C | 12 | 1.174 | 1.155–1.220 | .006 |
| D | 11 | 1.268 | 1.250–1.335 | .014 |
| H | 11 | 3.36 | 3.30 –3.45 | .02 |

Some of the specimens of February and March 1970 were maintained alive in separate containers at ambient sea water temperature at the Marine Sciences Research Laboratory and a few in a cold room at 0 C. For one reason or another all of these specimens died by August.

*Identification* — Since this species occurs at the same locations as *G. angulosus*, it was necessary to find reliable and easily discerned means of identification, particularly for small specimens. The status of this species and the criteria for its identification have been discussed by Bousfield (1956). In general most of the distinguishing characteristics outlined by Bousfield (1956) could be found readily in the specimens from Logy Bay, but difficulties were experienced with some (Table 1).

In addition, the two species when alive showed a striking difference in the coloration, which has not apparently been described previously. Whereas *G. angulosus* has an overall brownish-green color, *G. homari*, like so many other sublittoral animals in the Newfoundland region, is orange-red. This is the most useful character for distinguishing the two, but can only be applied to living specimens which are 8 mm or more in length. Preserved animals become bleached and may be difficult to identify. For the latter and for small animals the relative size of the eyes appears to be the most reliable character and can be employed even for quite small specimens. This is illustrated in Fig. 1.

TABLE 1. Comments on the characters which distinguish *Gammarellus homari* and *Gammarellus angulosus* (Bousfield 1956).

| Character | *G. angulosus* | *G. homari* |
|---|---|---|
| Size at maturity | 50% of females mature at 8.8 mm | females mature by 17 mm |
| Maximum size of females | 15 mm | 30 mm |
| Color | variegated, with an overall brownish-green color | variegated orange-red with white blotches; the redness is evident at the ends of appendages and somites at 8 mm |
| Size of eye | as in Bousfield (1956); see also Fig. 1 | |
| Antenna 1 | the number of segments in the accessory flagellum varies with size and is only distinctive for specimens of similar size | |
| Antenna 2 | the differences in the lengths of the 3rd and 4th segments of the peduncle are too slight to be of much use | |
| Coxal plate 1 | the differences in the anterior angles are difficult to appreciate unless specimens are compared directly | |
| Peraeopods 3–5 | the differences are slight | |
| Dorsal carination | the differences in carination of the peraeon are quantitative and not qualitative, and thus not very reliable: *G. angulosus* does have low carinations on its peraeon | |

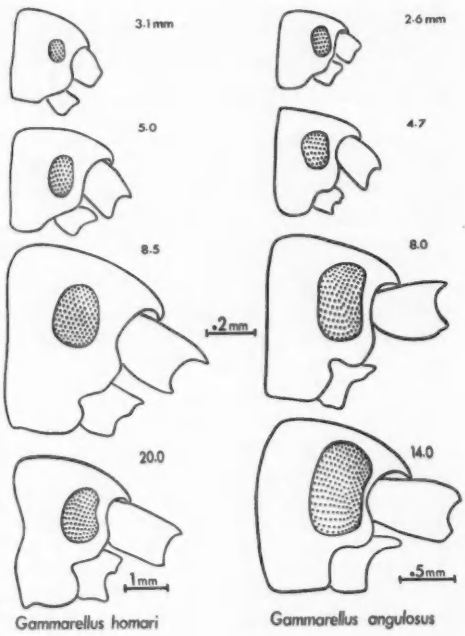


FIG. 1. Heads of *Gammarellus homari* and *Gammarellus angulosus* of varying size. They were drawn with the aid of a camera lucida.

*Distribution, habitat, and life cycle — Gammarellus homari* in the northwestern Atlantic is known from Ellesmere Island and northwest Greenland (Ohlin 1895) south to the St. Lawrence estuary and eastern Newfoundland (Bousfield 1956, present collections). Its distribution in the eastern Atlantic is uncertain due to confusion with *G. angulosus*, but according to Stephensen (1940) it has been reported from southern Norway north to Franz Joseph Land. In the light of this extensive northern distribution north of the Atlantic Ocean, it is somewhat surprising that it apparently does not occur between Novaya Zemlya and the Canadian Arctic Archipelago.

The habitat and life history of *G. homari* remain somewhat of a mystery. It appears to be a species of highly exposed rocky shore, where it coexists with *Gammarellus angulosus*. The collections made at Logy Bay are listed in Table 2. Small specimens were collected at LW in the spring and summer, although not usually in the company of *G. angulosus*. Large specimens were only rarely found at LW.

In February large adult females were found under rocks at a depth of 12–15 m. These were all ovigerous or spent and the young were released in March (Table 2). Later the females became scarce and finally disappeared from the shallow depths by April. Males have never been collected at these depths or elsewhere in the region of Logy Bay.

It seems likely that mating takes place in early winter, possibly in deeper water and that the ovigerous females migrate into shallow depths in the winter, remaining there until the young are released. The mature females then probably move back into deeper water, but the young remain near shore through the summer. The great size range of the mature females (17–30 mm) suggests that they can survive for several years and produce a number of broods.

Young hatched in February and maintained in running sea water reached a size of 5 mm in April and 8 mm in June.

The analysis of the females in Table 2 suggests that each produces a single brood each year. The ovigerous and spent females collected in the winter have only small oogonia in the ovaries, which indicates that the next brood will not be produced immediately. This was confirmed in the specimens maintained in the laboratory, in which oogonia did not enlarge during following months up to August. Instead, in subsequent molts during April–July, they lost their oostegite setae and entered the resting stage. Unfortunately, they were all killed in August due to an equipment failure. However, the single mature female collected in November had enlarged oogonia and probably would have mated shortly after it was caught. Although these observations are fragmentary and made over several years, they are all consistent with the hypothesis that each mature female produces a single brood of young in the fall or early winter and then enters a resting stage.

*Discussion — Gammarellus homari* is a sublittoral species in which the mature females (at least in the Newfoundland region) migrate into shallow water in the winter and release their young there in the spring. These large females are mainly hidden under rocks during the day but are more conspicuous at night (Dr J. M. Green personal communication). It is not known where or how the females spend the remainder of the year, or where the mature males live. The males have never been collected by diving to depths of 30 m, neither have they ever been dredged at depths down to 200 m, nor have been found in fish stomachs (personal observations). It is suggested, therefore, that they probably live under rocks in moderately deep water. A similar vertical migration has been observed in *G. carinatus* in the Black Sea (Greze 1964).

*G. homari* is a relatively large species compared to *G. angulosus* and has a more northern distribution. It apparently produces a single brood each year, with the young hatching in late winter and early

TABLE 2. Collections of *Gammarellus homari*.

| Date | Length (*mm*) | Sex | Embryonic Stage[a] | | | | | | | Stage of Oogonia and Oocytes in the Ovary | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | H | S | NS | 1 | 2 | 3 | 4 |
| Feb. 10,'70 | 17 | F | | | | | * | | | * | | | |
| | 17 | F | | | | | * | | | * | | | |
| | 18 | F | | | | | * | | | * | | | |
| | 18 | F | | | | | * | | | * | | | |
| | 19 | F | | | | | * | | | * | | | |
| | 19 | F | | | | | | * | | * | | | |
| | 19 | F | | | | * | | | | * | | | |
| | 20 | F | | | | | * | | | * | | | |
| | 20 | F | | | | | * | | | * | | | |
| | 22 | F | | | | | | * | | * | | | |
| | 24 | F | | | | | * | | | * | | | |
| | 30 | F | | | * | | | | | * | | | |
| Mar. 20,'70 | 19 | F | | | | | | * | | * | | | |
| | 24 | F | | | | | | * | | * | | | |
| | 25 | F | | | | | | * | | | | | |
| | 27 | F | | | | | | * | | * | | | |
| Apr. 18,'65 | 25 | F | | | | | | * | | * | | | |
| July 2,'67 | 7 | imm. | | | | | | | | | | | |
| | 8 | " | | | (12 individuals) | | | | | | | | |
| | 9 | " | | | (11 " ) | | | | | | | | |
| | 10 | " | | | | | | | | | | | |
| Sept. 23,'69 | 16 | F | | | | | | | * | * | | | |
| Nov. 23,'68 | 20 | F | | | | | | | * | | | | * |

[a]H = hatched; S = brood pouch empty; NS = no setae on oostegites and brood pouch empty.

spring. This is similar to what has been found in other northern amphipods, such as *Gammarus setosus* (Steele and Steele 1970) and *Gammarus wilkitzkii* (Barnard 1959).

*Acknowledgments* — The assistance of Dr J. M. Green, who made many of the subtidal collections and observations of *Gammarellus homari*, is gratefully acknowledged. This study was supported by the National Research Council of Canada.

BARNARD, J. L. 1959. Epipelagic and under-ice amphipoda of the central Arctic basin. *In* Scientific studies at Fletcher's Ice Island, T-3, 1952–1955. Geophys. Res. Pap. 63: 115–152.

BOUSFIELD, E. L. 1956. Malacostracan crustaceans from the shores of western Nova Scotia. Proc. N.S. Inst. Sci. 24(1): 25–38.

GREZE, I. I. 1964. Zhiznennyi tsikl bokoplava *Gammarellus carinatus* (Rathke) v Tchernom morye. Tr. Sevastopol Biol. Sta. Im A. D. Kovalenskogo Akad. Nauk SSSR 17: 293–303.

OHLIN, A. 1895. Bidrag till Kannedomen on Malakostrakfaunan i Baffin Bay och Smith Sound. Acta Univ. Lund. 31(6): 20–65.

STEELE, D. H., AND V. J. STEELE. 1969. The biology of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. I. *Gammarus duebeni* Lillj. Can. J. Zool. 47: 235–244.

1972. The biology of *Gammarellus angulosus* (Crustacea, Amphipoda) in the northwestern Atlantic. J. Fish. Res. Bd. Canada 29: 1337–1340.

STEELE, V. J., AND D. H. STEELE. 1970. The biology of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. II. *Gammarus setosus* Dementieva. Can. J. Zool. 48: 659–671.

STEPHENSEN, K. 1940. The Amphipoda of North Norway and Spitsbergen with adjacent waters. Fasc. 3, Tromsø Mus. Skr. 3: 279–362.

# Blood Concentrations of Tricaine Methane Sulphonate in Brook Trout, *Salvelinus fontinalis*, During Anesthetization, Branchial Irrigation, and Recovery

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HOUSTON, A. H., AND R. J. WOODS. 1972. Blood concentrations of tricaine methane sulphonate in brook trout, *Salvelinus fontinalis*, during anesthetization, branchial irrigation, and recovery. J. Fish. Res. Bd. Canada 29: 1344–1346.

Blood concentrations of tricaine methane sulphonate increased rapidly upon immersion of trout in 100 mg/liter solutions of the anesthetic at 3.5–5 C, and also rose significantly during irrigation for 15 min with either 50 or 100 mg/liter solutions. Clearance was rapid following transfer to running freshwater recovery tanks. Graphical estimates of 50 and 90% blood clearance times were 20 and 55 min, respectively.

HOUSTON, A. H., AND R. J. WOODS. 1972. Blood concentrations of tricaine methane sulphonate in brook trout, *Salvelinus fontinalis*, during anesthetization, branchial irrigation, and recovery. J. Fish. Res. Bd. Canada 29: 1344–1346.

Les concentrations sanguines de tricaïne méthane sulfonate augmentent rapidement lorsque des ombles de fontaine sont plongés dans des solutions de 100 mg/litre de l'anesthésique à 3.5–5 C. Elles s'élèvent aussi de façon significative lors de l'irrigation des branchies durant 15 min, avec des solutions de 50 ou 100 mg/litre. L'épuration est complétée peu après le transfert à l'eau douce courante dans les viviers de récupération. Le temps requis pour une épuration du sang de 50 et de 95%, estimé graphiquement, est de 20 et 55 min, respectivement.

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Tricaine methane sulphonate is, perhaps, the anesthetic most widely used in the preparation of teleosts for physiological study. It has been demonstrated that this agent produces a variety of effects beyond narcotization (e.g. Houston et al. 1971a; Willford 1970) and it may be presumed that these are, in part at least, concentration-dependent. Little is known, however, regarding the variations in blood concentration that occur during the experimental manipulation and recovery of specimens. The present study was accordingly undertaken, as a component of a program on problems related to experimental surgery in fishes (Houston et al. 1969, 1971a, b, 1972), with the specific aim of determining blood anesthetic concentrations in brook trout during anesthetization, branchial irrigation, and performance of routine preparative work, and subsequent recovery.

*Materials and methods* — Conditions of maintenance have been previously described (Houston et al. 1972). All groups of trout were held at 3.5 to 5 C for periods of not less than 3 weeks before use, and were tested at holding temperature.

To investigate anesthetic uptake specimens were individually anesthetized in aereated baths containing 10 liters of freshly-prepared 100 mg/liter solutions of Ayerst "Finquel®." Blood samples were drawn by caudal vein puncture into heparinized syringes after 5, 10, and 15 min exposures. In addition, sampling was also carried out upon attainment of the stage I plane of anesthesia (loss of equilibrium, although swimming movements and reactivity to mechanical stimuli persist) as defined by Schoettger and Julin (1967).

The effects of irrigation upon blood concentration were followed in fishes initially anesthetized for 15 min (100 mg/liter) and transferred to an operating assembly (Houston et al. 1969) on which the gills were irrigated with flows of approximately one liter/min for a further 15-min period. Two concentrations (50 and 100 mg/liter) were tested, the solutions being circulated through the irrigation device from a 10 liter reservoir of freshly-prepared, aereated anesthetic.

Clearance from the blood was studied by sampling animals previously anesthetized (100 mg/liter, 15 min) and irrigated (100 mg/liter, 15 min) and subsequently transferred to running freshwater recovery tanks at holding temperature for periods of 1, 2, 3, 6, 12, and 24 hr.

Finally, to establish blood concentrations of anesthetic at recovery, an additional group of trout were anesthetized (100 mg/liter, 15 min), shifted to recovery tanks

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and sampled immediately upon attainment of the upright position and apparently normal swimming movements. In this instance, and in the case of specimens sampled at the stage I level of anesthesia it was necessary to stun the animals prior to sampling.

As the principal objective of the study was to ascertain blood anesthetic concentrations under conditions comparable to those during actual experimental preparation, the specimens used in irrigation and recovery studies were subjected to the procedures most frequently carried out in this laboratory. This involved emplacement of dorsal aortic, cleithral, buccal, and urinary bladder catheters and a set of three ECG pin electrodes (Houston et al. 1971a, 1972; Houston 1971). While all usual precautions were followed no attempt was made to ensure that the resulting preparation was operational. All aortic catheters, for example, were cut short and plugged upon completion of the procedure. It was not possible to carry out all phases of this aspect of the study upon specimens irrigated with 50 mg/liter solution. This concentration, while it ensured quiescence, was generally inadequate for maintenance of surgical planes of anesthesia.

The Bratton–Marshall technique, as modified by Walker and Schoettger (1967a) was used for determination of blood anesthetic concentrations. Comparisons of results were made by Student's *t*-test, and significance attached to differences at $P \geq 0.05$.

The weights and numbers of specimens used are given in Table 1.

*Results and discussion* — Values for animals sampled at the stage I level of anesthesia indicated that anesthetic uptake was extremely rapid; less than 2 min being required to reach this stage (Table 1). Blood concentrations (56.2 ± 8.42 mg/liter) were, however, below those reported by Hunn (1970) for rainbow trout under somewhat similar conditions. No significant change in concentration was observed during continued exposure, a situation which also contrasts with that described by Hunn. It is, however, apparent that anesthetic uptake does continue, for both brain and muscle levels increase significantly during the post-stage I period despite apparent stabilization of blood concentrations (Hunn 1970; A. H. Houston and R. J. Woods unpublished data).

Correlation analysis carried out on data for the group of 30 animals exposed for 15 min revealed no significant correlation between either body

TABLE 1. Mean blood concentrations of tricaine methane sulphonate in brook trout exposed to 100 mg/liter solution and sampled during induction of anesthesia, branchial irrigation, and recovery in fresh water.

| Phase of study | Treatment and sampling | Temp. (*C*) | Time to stage I or recovery (*sec*) | Blood anesthetic level (*mg/liter*) | Weight (*g*) of fish |
|---|---|---|---|---|---|
| A. Induction of anesthesia | Exposure: | | | | |
| | to stage I plane of anesthesia | 5 | 113±5.5[a] | 56.2±8.42 | 370.8±17.0g |
| | for 5 min | 4.5 | | 52.8±3.73 | 422.1±33.4g |
| | for 10 min | 4 | | 55.5±2.98 | 433.9±26.1g |
| | for 15 min | 5 | | 55.7±2.11 | 412.2±16.9g[b] |
| B. Branchial irrigation | Exposure for 15 min followed by 15-min irrigation with solution of: | | | | |
| | 50 mg/liter | 3.5 | | 67.1±3.74 | 392.3±23.4g |
| | 100 mg/liter | 3.5 | | 114.2±4.47 | 394.2±33.0g |
| C. Clearance of anesthetic | Exposure for 15-min and irrigation with 100 mg/liter solution for 15 min, followed by transfer to recovery tanks for: | | | | |
| | 1 hr | 5 | | 16.7±0.72 | 428.5±42.8g |
| | 2 hr | 5 | | 6.2±0.63 | 432.9±20.9g |
| | 3 hr | 5 | | 6.2±0.60 | 366.4±44.2g |
| | 6 hr | 5 | | 4.5±0.57 | 453.8±37.6g |
| | 12 hr | 5 | | 4.3±1.31 | 415.3±41.8g |
| | 24 hr | 5 | | 4.1±0.72 | 432.4±36.9g |
| D. Blood anesthetic level at attainment of equilibrium | Exposure for 15 min followed by transfer to recovery tank. | 3.5 | 374±53.2 | 25.2±2.01 | 463.9±35.1g |

[a]One standard error of mean.
[b]30 fish in test; 10 in all others.

weight or sex and blood anesthetic concentration. A significant negative correlation was observed between body weight and the time required for anesthetization to the Stage I level ($r = -0.67$, $P > 0.05$). While similar correlations have been observed in rainbow trout (A. H. Houston and R. J. Woods unpublished data) no explanation for this phenomenon can be offered at the present time.

Irrigation with 50 mg/liter solutions induced a rise in anesthetic concentration ($P > 0.05$) but, as was previously noted, this was inadequate for prolonged manipulation of the specimens. Irrigation with 100 mg/liter solution, which did permit extensive work, resulted in blood concentrations which exceeded both that at the end of the initial 15-min exposure ($P > 0.01$) and the concentration of the irrigating solution ($P > 0.05$). The latter observation raises a number of questions regarding the movement of anesthetic within the animal and these will be considered in a subsequent report. It also suggests the desirability of ascertaining irrigation concentrations which will adequately maintain anesthesia without leading to excessive accumulations of drugs that must subsequently be cleared during the recovery process.

Fish in group C regained equilibrium within 5 to 10 min. In the trout exposed for 15 min to 100 mg/liter anesthetic solution and then transferred to the recovery tank (group D), the attainment of equilibrium was associated with a mean blood concentration of $25.2 \pm 2.01$ mg/liter. The regression of blood concentration against the time required to reach this stage of recovery had a slope which did not differ significantly from zero, suggesting that recovery is associated with a decline in blood concentration below some critical level. In any event the anesthetic was rapidly cleared from the blood upon transfer to fresh water. No significant decreases were observed after 6 hr, and the values obtained at 6, 12, and 24 hr did not differ significantly from the background primary aromatic amine concentrations observed in previously unanesthetized trout ($4.2 \pm 0.5$ mg/liter). Finally, a plot of the logarithm of concentration versus time yielded graphical estimates for 50 and 90% blood clearance times of 20 and 55 min, respectively, these values being in good agreement with those reported by Walker and Schoettger (1967b) for other salmonids.

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HOUSTON, A. H. 1971. A simple improvement in the vascular catheterization procedure for salmonid and other teleost fishes. J. Fish. Res. Bd. Canada 28: 781–783.

HOUSTON, A. H., C. L. CZERWINSKI, AND R. J. WOODS. 1972. Cardiovascular-respiratory activity in brook trout (*Salvelinus fontinalis*) and carp (*Cyprinus carpio*) during recovery from anesthesia and surgery. J. Fish. Res. Bd. Canada (In press)

HOUSTON, A. H., M. A. DEWILDE, AND J. A. MADDEN. 1969. Some physiological consequences of aortic catheterization in the brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Canada 26: 1847–1856.

HOUSTON, A. H., J. A. MADDEN, R. J. WOODS, AND H. M. MILES. 1971a. Some physiological effects of handling and tricaine methanesulphonate anesthetization upon the brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Canada 28: 625–633.

1971b. Variations in the blood and tissue chemistry of brook trout, *Salvelinus fontinalis*, subsequent to handling, anesthesia and surgery. J. Fish. Res. Bd. Canada 28: 635–642.

HUNN, J. B. 1970. Dynamics of MS-222 in the blood and brain of freshwater fishes during anesthesia. U.S. Bur. Sport Fish. Wildlife Resour. Publ. 42: 1–8.

SCHOETTGER, R. A., AND A. M. JULIN. 1967. Efficacy of MS-222 as an anesthetic on four salmonids. U.S. Bur. Sport Fish. Wildlife Resour. Publ. 13: 1–15.

WALKER, C. R., AND R. A. SCHOETTGER. 1967a. Method for determining MS-222 residues in fish. U.S. Bur. Sport Fish. Wildlife Resour. Publ. 14: 1–10.

1967b. Residues of MS-222 in four salmonids following anesthesia. U.S. Bur. Sport Fish. Wildlife Resour. Publ. 15: 1–11.

WILLFORD, W. A. 1970. Effect of MS-222 on electrolyte and water content in the brain of rainbow trout. U.S. Bur. Sport Fish. Wildlife Resour. Publ. 43: 1–7.

# Bunker C Oil in Sediments and Benthic Animals from Shallow Depths in Chedabucto Bay, N.S.

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SCARRATT, D. J., AND V. ZITKO. 1972. Bunker C oil in sediments and benthic animals from shallow depths in Chedabucto Bay, N.S. J. Fish. Res. Bd. Canada 29: 1347–1350.

Soft sediments showed considerable fluctuations in Bunker C oil content but little evidence of diminution of Bunker C concentration in the 26 months following the wreck of the tanker *Arrow*. Coarse sediment samples and most benthic species showed maximum oil concentrations about 1 year after the wreck and some reduction since then. Herbivorous or browsing species had higher oil content than carnivorous or omnivorous species. There is evidence from fluorescence emission spectra that some carnivorous or omnivorous species are able to assimilate and partly metabolize Bunker C oil, but no evidence that Bunker C or its fluorescent derivatives and fractions were being concentrated in higher parts of the food chain.

SCARRATT, D. J., AND V. ZITKO. 1972. Bunker C oil in sediments and benthic animals from shallow depths in Chedabucto Bay, N.S. J. Fish. Res. Bd. Canada 29: 1347–1350.

La teneur en huile lourde C des sédiments mous a varié considérablement au cours des 26 mois qui ont suivi l'échouement du pétrolier *Arrow*, sans qu'il y ait eu signe de diminution. Les échantillons de sédiments à gros grain et la plupart des espèces benthiques ont marqué un maximum de concentration d'huile environ un an après l'échouement et une certaine diminution depuis ce temps. Les espèces herbivores ou vagiles ont une teneur en huile supérieure à celle des espèces carnivores ou omnivores. Les spectres d'émission de fluorescence démontrent l'aptitude de certaines espèces carnivores ou omnivores à assimiler et métaboliser partiellement l'huile lourde C, mais ne fournissent aucune preuve de la concentration de l'huile elle-même, ou de ses fractions ou dérivés fluorescents dans les maillons supérieurs de la chaîne alimentaire.

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FOLLOWING the wreck of the *Arrow* on February 4, 1970, and the subsequent release of about two thirds of the cargo of 108,000 barrels of Bunker C fuel oil, a survey was made of the distribution of oil under water in Chedabucto Bay and its possible effects on the sublittoral biota. Initial observations have been summarized (Anon. 1970; Scarratt et al. 1970; and Zitko 1970).

Two of the 35 sites initially examined were selected for continued monitoring. At Beach Point, Jerseyman Island (Fig. 1) the sea bed was a steeply pitched mud bank. Shortly after the wreck, globules and pats of oil ballasted with sand and pebbles had been found in depths between LWM and 12 m at this site, but these disappeared with the advent of warmer water. The benthos was sparse but a number of representative specimens could generally be collected. Collections were made at 3 and 12 m.

At Crichton Island on the exposed south side facing the wreck, the substrate was of rock and large boulders with occasional patches of well sorted fine gravel with abundant macroflora and fauna. Collections were made at 3.5 and 7 m depths.

This paper describes the Bunker C oil content of sediment and benthic organisms taken from these two stations at irregular intervals during the 26 months following the wreck. Some of the data have been published elsewhere (Zitko 1970) but for completeness are represented here as part of a complete series.

*Methods* — Soft sediments were sampled by divers thrusting open-ended thin wall plexiglas tubes (about 25 cm long × 5 cm internal diameter) vertically into the mud. A rubber stopper was manoeuvered into the lower end of tube which was then withdrawn from the sediment, the upper stopper inserted, and the whole brought undisturbed to the surface and frozen. For analysis the cores (12–15 cm long) were cut into three horizontal sections which were analysed separately.

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Gravel areas were generally too thin for coring and samples were collected by scraping into a polythene bag. There is thus no division of gravel samples into strata. Samples of common animals (1–3) were simply picked off the bottom at each location, brought to the surface, and frozen. Control samples were obtained from Passamaquoddy Bay, near the St. Andrews laboratory, and from near Pictou, N.S. Weighed amounts of sediment or core material were extracted with hexane (Fisher H334) and the concentration of Bunker C oil determined by U.V. spectrophotometry. Determination of oil content of whole animals or separate organs has been described by Zitko (1970).

*Results* — Samples taken at Beach Point, Jerseyman Island, showed some fluctuations in oil content during the 26-month period ending April 1972, but there is no evidence of any diminution (Table 1). There is some suggestion that in shallow water heavily oiled sediments may be becoming overlaid by slightly cleaner material, however, after 2 years, the surface layers still contained in excess of 300 µg/g of Bunker C oil. Apart from the initial high concentration in March, 1970, sediments at 12 m had slightly less oil than those at 3 m. The fluorescence emission spectrum of the middle and lower levels of one intermediate early depth core (not listed in Table 1) was different from that of *Arrow* Bunker C, suggesting that the area had received earlier contamination from a different source.

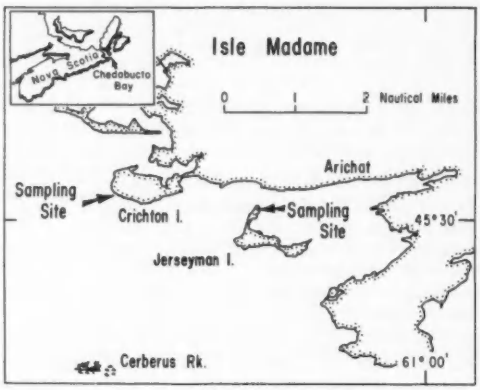


FIG. 1. Underwater sampling stations, Chedabucto Bay.

At Crichton Island gravel samples taken after one year at 3.5 and 7 m depths yielded 38 and 78 µg/g Bunker C, respectively, and 5 and 11 µg/g after 26 months. Although there were no earlier samples from this area, the concentrations are higher after 12 months than those found in comparable gravel samples taken in Chedabucto Bay in February 1970 (0.2–3.6 µg/g) and the Bunker C equivalent of the soft sediment cores taken in 5 m of water off Pictou. Oil content of gravel samples after 2 years is only marginally higher than in controls.

*Oil in fauna* — There was no visible evidence that Bunker C had contributed to the deaths of any animals below LWM, although analysis showed many species were ingesting it. Bunker C oil was detected qualitatively in clams (*Mya arenaria*) and scallops (*Placopecten magellanicus*) which are of

TABLE 1. Bunker C oil in sediment cores. *µg/g* wet weight.

| Depth | 1970 Mar. | May | June | July | Oct. | 1971 Feb. | 1972 Apr. | Part of core |
|---|---|---|---|---|---|---|---|---|
| | | | | *Jerseyman Island* | | | | |
| 3 m | 69 | 189 | 34 | 481 | 778 | 420 | 366 | top |
| | 57 | 12 | 28 | 773 | 41 | | 346 | middle |
| | 63 | 6 | 70 | 244 | 5 | 625 | 1258 | bottom |
| 12 m | 1240 | 72 | 11 | 99 | 190 | 145 | 280 | top |
| | 156 | 10 | 24 | 5 | 102 | | 17 | middle |
| | 332 | 4 | 21 | 1 | 53 | 30 | 2 | bottom |
| | | | | *Control samples, Pictou, N.S.* | | | | |
| 5 m | | | | 5[a]3[a] | | | | top |
| | | | | 7[a]1[a] | | | | middle |
| | | | | 8[a]0.5[a] | | | | bottom |

[a]No fluorescence emission maximum at 360 *nm*.

minor commercial importance in the area. Scallops showed positive results for mantle, digestive gland, adductor, and gonad, showing that they were assimilating ingested Bunker C. The periwinkle (*Littorina littorea*) on the other hand appears to pass Bunker C through the intestine unabsorbed. Fluorescence was confined to gut contents and faeces. Extracts of digestive gland, foot and mantle showed no fluorescence.

Five species were sampled fairly consistently at both sample sites during the 26-month period (Table 2). Of the herbivorous or filter feeding species, periwinkles showed highest concentrations with values ranging between 27 and 600 μg/g wet weight. Mussels (*Modiolus modiolus*) had concentrations between 21 and 372 μg/g. Sea urchins (*Strongylocentrotus droebachiensis*) had concentrations between 17 and 94 μg/g. In all instances controls had background fluorescence from which a Bunker C equivalent could be calculated but emission spectra were without the characteristic Bunker C emission peak at 360 nm. By April 1972 these three species showed considerable reduction in Bunker C content, sea urchins in particular being at or below control level of background fluorescence. Among the predatory species some starfish (*Asterias vulgaris*) had Bunker C concentrations ranging between 28 and 400 μg/g. Others however had background fluorescence equivalent to Bunker C concentrations between 14 and 52 μg/g (control:11) but without the characteristic emission peak at 360 nm. Crabs (*Cancer irroratus*)

TABLE 2. Bunker C oil in common benthic species. *μg/g* wet weight. Control concentrations in brackets.

| Species | Location | Depth (*m*) | 1970 Mar. | May | June | July | Oct. | 1971 Feb. | 1972 Apr. |
|---|---|---|---|---|---|---|---|---|---|
| Periwinkle (11[a]) | Crichton Is. | 3.5 | 44 | 102 | 97 | 57 | 150 | 123 | 27 |
| (*Littorina littorea*) | | 7 | 31 | 556 | 219 | 66 | 98 | 81 | |
| | Jerseyman Is. | 3 | 208 | | 604 | 231 | 586 | 423 | 51 |
| | | 12 | 304 | 98 | | | | | 112 |
| Mussels (3[a]) | Crichton Is. | 3.5 | 123 | 141 | 98 | 103 | | 190 | 41 |
| (*Modiolus modiolus*) | | 7 | | | 132 | 77 | 77 | 98 | 36[a] |
| | Jerseyman Is. | 3 | 44 | | | 21 | | | |
| | | 12 | 31 | 372 | | 39 | | | |
| Sea Urchins (22[a]) | Crichton Is. | 3.5 | | | 40 | 56 | | 47 | 21 |
| (*Strongylocentrotus droebachiensis*) | | 7 | | | 94 | | | 36 | 17 |
| Starfish (11[a]) | Crichton Is. | 3.5 | | 158 | 399 | | 251 | 115 | 100,30 |
| (*Asterias vulgaris*) | | 7.0 | | 31 | 16[a] | | 29[a] | 52[a] | 15[a] |
| | Jerseyman Is. | 12 | | 14[a] | 28 | | | 64 | 36 |
| Crab (7[a]) | Crichton Is. | 3.5 | 28[a] | 28 | 118[a] | 61[a] | | 25[a] | |
| (*Cancer irroratus*) | | 7 | 28[a] | | | 17[a] | | | 7[a]18[a] |
| | Jerseyman Is. | 3 | | 64[a] | | | 59[a] | | |
| | | 12 | | | 53[a] | | | | |

[a]No fluorescence emission maximum at 360 *nm*.

TABLE 3. Bunker C oil in lobsters, *Homarus americanus*. *μg/g* wet weight.

| Tissue | Pictou control | Lab control | Lab exposure[a] | Chedabucto Bay May/70 | Chedabucto Bay July/70 |
|---|---|---|---|---|---|
| Abdominal muscle | 5[b] | 3[b] | 137 | 1[b] | 4[b] |
| Claw muscle | 4[b] | 4[b] | 33 | 2[b] | 3[b] |
| Stomach | 19[b] | 149[b] | 2840 | 15[b] | 230[b] |
| Intestine | 57[b] | 116[b] | 1810 | 103[b] | 130[b] |

[a]6½ days in 10,000 *mg/liter* Bunker C at 4 C followed by 7 days in running water.
[b]No maximum at 360 *nm*.

with one exception had background fluorescence 2½ to 17 times that of the control. The exception, a small crab collected April 1972, had the same Bunker C equivalent concentration as the control. Only crabs with high Bunker C content had emission maxima at 360 nm (Table 2).

Lobsters (*Homarus americanus*) are not highly contaminated by oil. Two sampled in spring and summer of 1970 showed fluorescence only marginally higher than samples from uncontaminated water at Pictou, N.S., and St. Andrews, N.B., and considerably less than that from lobsters deliberately exposed to Bunker C (Table 3).

The absence of fluorescence emission maxima at 360 nm in species showing high background fluorescence suggests strongly that fluorescent compounds from ingested Bunker C or from Bunker C contaminated prey are either metabolized or bound up in the tissues and different fluorescent compounds generated in the process. Other species showing high background fluorescence with no emission maximum at 360 nm were toad crabs (*Hyas araneus*): 24 µg/g; and hermit crabs (*Pagurus bernhardus*): 114 and 43 µg/g in 1970 and as low as 18 in 1972. Control for hermit crabs was two.

Some emission spectra showed maxima at 360 nm but had spectra differing from standards. It is probably safe to assume that part of the Bunker C may have been assimilated and metabolized.

*Discussion and conclusions* — Bunker C oil was still detectable in sediments and many animals 26 months after *Arrow* stranded. Generally samples from shallow stations contained more oil than samples from deeper stations. There is still (April 1972) a large amount of oil on the beaches. Most of this is now bound up as a matrix with beach material and its rate of erosion off the beach and incorporation into subtidal sediments must be considerably reduced. There is no visible evidence of oil in the water column and recent work by Gordon and Michalik (1971) suggests that the waters of Chedabucto Bay are now clean. The period of maximum concentrations of oil in the fauna appears to be past, although it will probably be several years before fluorescence emission spectra of all species from all parts of Chedabucto Bay approach those of specimens from uncontaminated areas.

The generally low Bunker C content of carnivorous species compared with browsing species suggests strongly that Bunker C is not being concentrated in the higher parts of the food chain, although the possibility that certain fractions not detectable by fluorescence techniques are being concentrated cannot be ruled out.

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ANON. 1970. Report of the task force - operation oil. Vol. II. Issued by the Ministry of Transport, Ottawa, Ont. 104 p.

GORDON, D. C., JR., AND P. A. MICHALIK. 1971. Concentration of bunker C fuel oil in the waters of Chedabucto Bay, April 1971. J. Fish. Res. Bd. Canada 28: 1912–1914.

SCARRATT, D. J., J. B. SPRAGUE, D. G. WILDER, V. ZITKO, AND J. M. ANDERSON. 1970. Some biological and chemical investigations of a major winter oil spill on the Canadian east coast. ICES Fish. Improv. Comm. Rep. 14: 8 p.

ZITKO, V. 1970. Determination of residual fuel oil contamination in aquatic animals. Bull. Environ. Contam. Toxicol. 5: 559–564.

# Electrical Responses of the Olfactory Bulb of Pacific Salmon *Oncorhynchus nerka* and *Oncorhynchus kisutch*

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HARA, T. J. 1972. Electrical responses of the olfactory bulb of Pacific salmon *Oncorhynchus nerka* and *Oncorhynchus kisutch*. J. Fish. Res. Bd. Canada 29: 1351–1355.

Olfactory bulbar electrical responses elicited by nasal infusion of food extracts, hand rinse, and several amino acid solutions were studied in sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon from several different sources. Comparison was also made in rainbow trout (*Salmo gairdneri*). Significant variations in the olfactory bulbar responses were observed among different species and even among the same species from different sources. The estimated lowest threshold concentration for L-serine was between $10^{-6}$ and $10^{-7}$ M in salmon. Olfactory responses of rainbow trout were always several times larger than those of salmon.

Pretreatment of nasal cavity with $10^{-4}$ M solutions of $HgCl_2$ or $CuSO_4$ (27 or 16 mg/liter, respectively) for 10 sec completely blocked the olfactory responses in all fish species studied. The effect was reversible; the responses returned to their original level by rinsing. Exposure of fish to 0.1 mg/liter $HgCl_2$ for up to 3 days reduced the olfactory responses to 40–10% of those of normal fish. $CuSO_4$ was the more toxic.

HARA, T. J. 1972. Electrical responses of the olfactory bulb of Pacific salmon *Oncorhynchus nerka* and *Oncorhynchus kisutch*. J. Fish. Res. Bd. Canada 29: 1351–1355.

Nous avons étudié la production de courants électriques par le bulbe olfactif du saumon nerka (*Oncorhynchus nerka*) et du saumon coho (*O. kisutch*) provenant de plusieurs sources différentes, à la suite de l'introduction, dans les sacs olfactifs, d'extraits d'aliments, d'eau de rinçage des mains et de plusieurs solutions d'acides aminés. Nous avons également comparé les réponses de la truite arc-en-ciel (*Salmo gairdneri*). La réponse du bulbe olfactif diffère nettement entre les espèces et même entre les individus d'une même espèce provenant de sources différentes. L'estimation de la concentration de seuil minimale pour la L-sérine se situe entre $10^{-6}$ et $10^{-7}$ M chez les saumons. La réponse olfactive des truites arc-en-ciel est toujours plusieurs fois plus forte que celle des saumons.

Le prétraitement du sac nasal avec une solution $10^{-4}$ M de $HgCl_2$ ou de $CuSO_4$ (27 ou 16 mg/litre respectivement) pendant 10 secondes bloque complètement la réponse olfactive de toutes les espèces étudiées. L'effet est réversible, et le rinçage rétablit la réponse à son niveau originel. L'exposition des poissons à 0.1 mg/litre de $HgCl_2$ durant une période allant jusqu'à trois jours abaisse la réponse olfactive à 40–10% de celle des poissons normaux. $CuSO_4$ est le plus toxique des deux.

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THE importance of the sense of smell in many facets of the life of salmon has been generally accepted from behavioral and electrophysiological experiments (see reviews by Hara 1970, 1971; Hasler 1966). It has long been known that Pacific salmon respond to a repellent in mammalian skin at extremely high dilution, and that amino acid L-serine is one of the active compounds of the repellent (Idler et al. 1956; Brett and MacKinnon 1954). The involvement of the sense of smell in this response has been suggested by these authors.

The investigation was undertaken to determine by electrophysiological methods the sensitivity and range of olfactory response of young Pacific salmon and to test possible effects produced by mercury and copper salts.

*Materials and methods* — Sockeye salmon, *Oncorhynchus nerka*, were obtained from the Nanaimo Biological Station and Rosewall Creek, both located in Vancouver Island, and also from Cultus Lake, B.C. Coho salmon, *O. kisutch*, were obtained from Rosewall Creek. Most fish were in their second year and ranged from 19.6 to 23.5 cm. For comparison, rainbow trout, *Salmo gairdneri*, of similar size were also obtained from West Vancouver, B.C. They were held in a fiberglass tank, which was provided with a continuous flow of water (temperature 14–16 C) prior to use.

Operative procedures and recording techniques employed were essentially similar to those described previously (Hara 1972). As test stimulants, food extracts,

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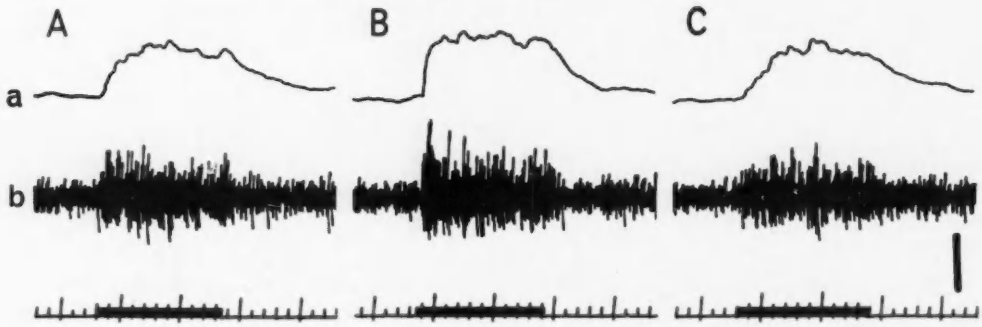


FIG. 1. Typical responses recorded from the olfactory bulb of coho salmon. (a), amplitude–frequency integration of (b), oscillatory bulbar response. A, hand rinse; B, food extracts; C, $10^{-4}$ M L-serine. The period of stimulation and time scale indicating 1-sec intervals are shown below each record. Calibration 50 $\mu$V.

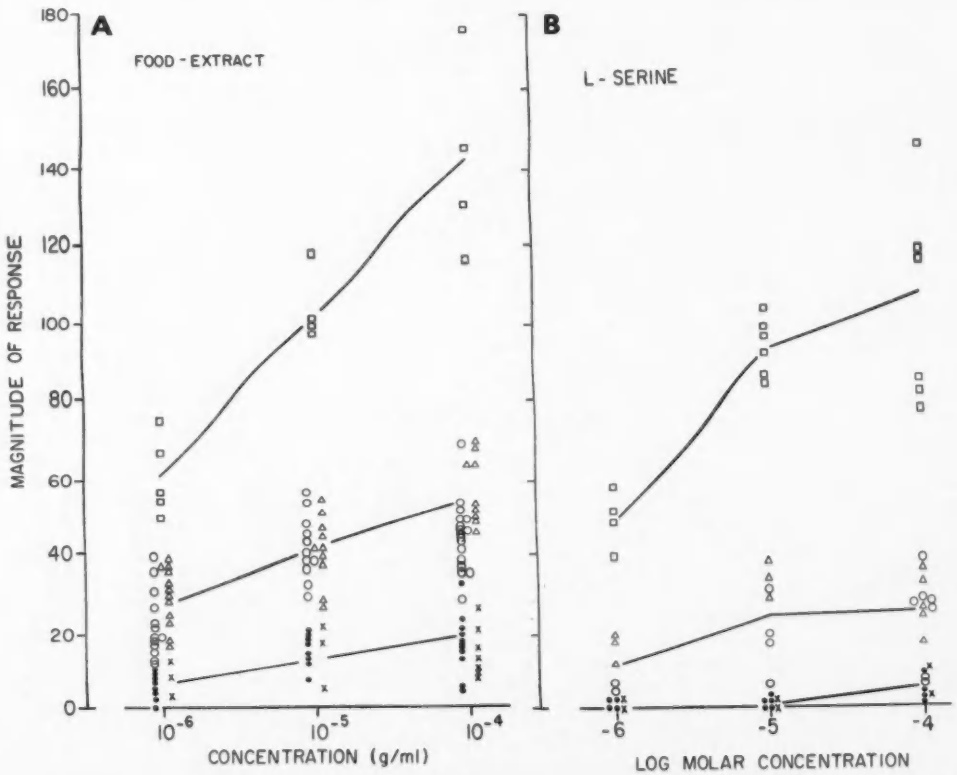


FIG. 2. Concentration–response relations showing the variations among all fish groups studied. A, food extracts; B, L-serine. Squares, rainbow trout; triangles, coho salmon; crosses, sockeye from the Biological Station; open circles, sockeye from Cultus Lake; closed circles, sockeye from Rosewall Creek.

hand rinse, and amino acids serine, alanine, and methionine, of both L- and D- forms, were used. Aqueous extracts of food were prepared by suspending 1-g food pellets (J. R. Clark Co., Salt Lake City, Utah) in 100 ml of distilled water. The suspension was thoroughly mixed for 5 min, then after filtering stored as a stock solution. Hand rinse was freshly prepared daily by dipping a forefinger in 100 ml of water for 10 sec. The amino acid L-serine was chosen as test stimulant since it is known to be one of the active components of the skin rinse of mammalian predators causing avoidance reactions in migrating salmon (Idler et al. 1956; Brett and MacKinnon 1954). L-Alanine and L-methionine are also known to be effective olfactory stimulants in fish (Hara 1972; Suzuki and Tucker 1971; Sutterlin and Sutterlin 1970). Aqueous solutions of chemicals were prepared with distilled water at the concentration of $10^{-3}$M, and all subsequent dilutions were made daily with the same water as that which perfused the gills and nares.

*Results* — When the nasal sac of salmon is stimulated with water containing odorous stimulants a high amplitude electroencephalographic (EEG) response, oscillatory in nature, is recorded from the olfactory bulb. Typical responses induced in the olfactory bulb of coho salmon by application of hand rinse, food extracts, and $10^{-4}$M L-serine are shown in Fig. 1. In all subsequent experiments magnitude of the responses are expressed in terms of the total integrated response (Fig. 1a) in arbitrary unit during stimulation. The lowest estimated threshold concentration for L-serine and two other effective amino acids ranges between $10^{-6}$ and $10^{-7}$M in coho salmon. It is higher in sockeye salmon.

In the course of experiments, it was noticed that significant variations exist in the olfactory responses among the different fish species studied and also among the same species from different places. The concentration–response relations for food extracts and L-serine in all fish groups tested are plotted in Fig. 2. Three groups can be clearly distinguished; (1) rainbow trout — largest responses, (2) sockeye salmon from the Biological Station and Rosewall Creek — smallest responses, and (3) coho salmon and sockeye salmon from Cultus Lake — intermediate responses. This relation remains fairly consistent for all other stimulants tested (Fig. 3). However, the order of magnitude of the response to equivalent molar concentration of amino acids is different from species to species. The effectiveness of D-isomers was always less than that of the L-forms.

*Effects of heavy metals* — Pretreatment of the nasal cavity with the solution of $10^{-4}$M (27 mg/liter) $HgCl_2$ for 10 sec blocked almost completely the responses to all stimulants used. The effect of a single treatment with mercury on the bulbar re-

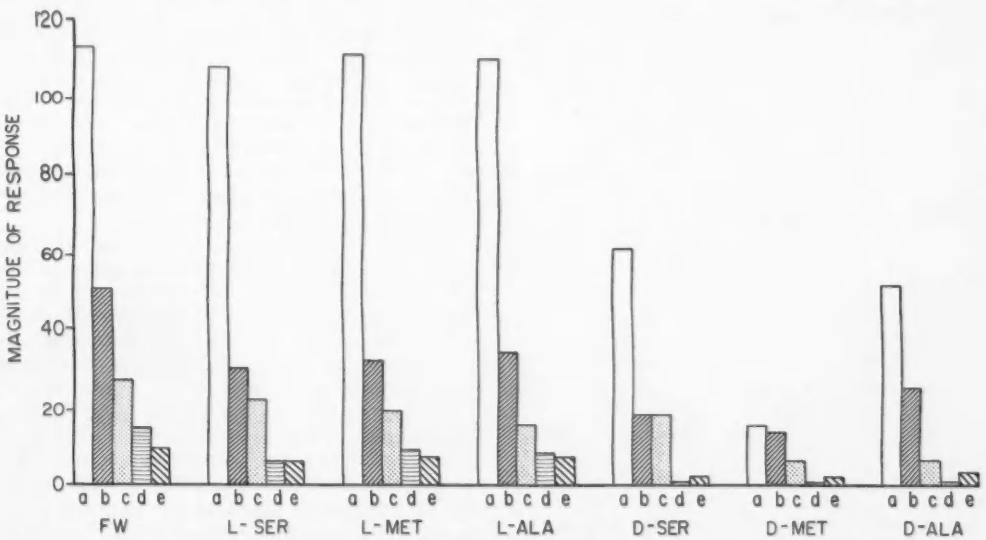


FIG. 3. Responses to seven different odorous stimulants in five different fish groups. a, rainbow trout; b, coho; c, sockeye from Cultus Lake; d, sockeye from Rosewall Creek; e, sockeye from the Biological Station. Amino acids were all tested at $10^{-4}$ M.

sponse was reversible; the response recovered to its original level after 20–30 min rinsing with fresh water. The blocking effect of $CuSO_4$, at the same concentration, was stronger than that of $HgCl_2$ and a longer period was required for the recovery of the response.

The effect of longer exposure of fish to these heavy metals was tested by continuous introduction of the appropriate solution into an experimental tank (polyethylene, 20×45×45 cm) by use of a peristaltic pump at the final concentration of 0.1 mg/liter for up to 3 days. In Fig. 4 magnitude of responses is plotted against three different concentrations of food extracts in normal and $Hg^{++}$-exposed groups of sockeye and coho salmon. Experiments with other stimulants indicate that the blocking effect of $HgCl_2$ is more apparent in the response of single chemical stimulus such as L-serine than to a compound stimulus like food extracts or hand rinse. Copper sulphate was more toxic than $HgCl_2$. No response to any kind of stimulant tested was obtained from the fish exposed to 0.1 mg/liter $CuSO_4$ for more than 12 hr.

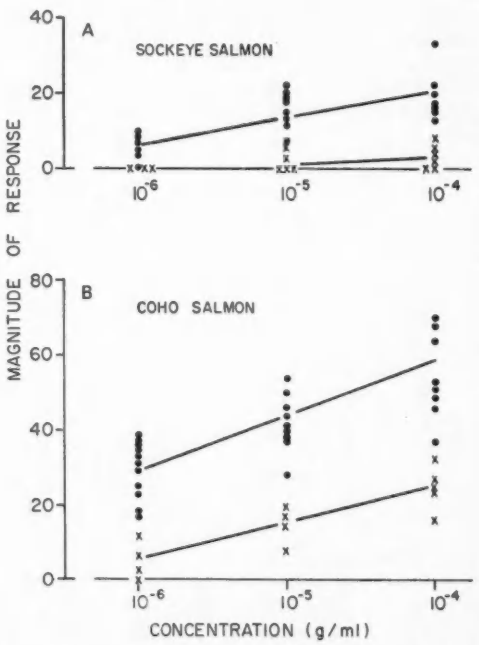


FIG. 4. Effects on the olfactory responses of $Hg^{++}$-exposure in sockeye (A) and coho (B) salmon. Food extracts are used as odorant stimuli. Circles and crosses indicate normal and $Hg^{++}$-treated (0.1 mg/liter for up to 3 days) fish, respectively.

*Discussion* — Behavioral experiments have shown that amino acid L-serine is one of the active components of mammalian skin factor. The D-isomer showed no activity. L-cysteine, L-leucine, L-isoleucine, L-valine, L-methionine, and L-cystine were all inactive (Idler et al. 1956). In the present experiment, however, L-serine was not especially stimulatory in relation to several other amino acids. Both L-methionine and L-alanine were observed to be slightly more active than L-serine in all fish groups except one (sockeye salmon from Cultus Lake). Discriminatory capacity of the young may be different from that of adults. No experimental evidence has been produced as to whether young salmon respond to the so-called mammalian skin factor, except for the statement by Wright (1964) that "...It is interesting that young fish on their way *downstream* to the sea do not show the reaction..." The threshold concentration estimated for these effective amino acids is a little higher in salmon than in rainbow trout. The threshold values for rainbow trout in the present experiment is very similar to those previously obtained (Hara 1972). The D-isomers were also fairly stimulatory; the relative effectiveness of D-serine, D-alanine, and D-methionine in coho salmon were 60, 75, and 40%, respectively, of their L-forms. There is likely to be a slight difference among the ranks of the relative effectiveness of amino acids in different fish species tested, possibly reflecting a species specificity. This view is supported by the recent studies on the olfactory receptor responses to amino acids in Atlantic salmon (Sutterlin and Sutterlin 1971) and catfish (Suzuki and Tucker 1971). Detailed discussion on the olfactory bulbar response to amino acids in rainbow trout will be found elsewhere (Hara 1972). It still remains to be determined whether or not free amino acids themselves have any specific significance in olfactorily mediated behavior of fishes.

The lowest threshold concentration of food extracts capable of eliciting olfactory response was estimated to be between $10^{-7}$ and $10^{-8}$ mg/ml for salmon. It was a little lower for rainbow trout. These figures were of nearly the same order as obtained for juvenile sockeye salmon by behavioral tests (McBride et al. 1962).

The point that responsiveness varied among populations of the same species obtained from different sources is significant, since all the experimental conditions were practically the same except for the time period necessary for transporting the experimental fishes from different places. The variation may be due to their genetic background and different cultural condition, giving the fish a different sensory background. The responsiveness of

rainbow trout, tested for comparison, was comparable to that obtained earlier in other series of experiments (Hara 1972).

The effects of heavy metals as blocking agents have been demonstrated in a number of chemosensitive systems (Sutterlin and Sutterlin 1970, 1971; Hidaka 1970; Hidaka and Yokota 1967; Yur'eva 1957). The rapid depression or complete blockage of the olfactory bulbar response to all stimulants tested by application of $HgCl_2$ or $CuSO_4$ to the nose, and its rapid recovery by rinsing suggest that the reaction may take place on the receptor membrane, perhaps on the receptor site. Mercuric ion is known to tie up sulfhydryl groups of proteins whereas cupric ion ties up amino groups. The blocking effect observed here may be due to interference with either receptor sites or enzymatic components of the receptor membranes.

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BRETT, J. R., AND D. MACKINNON. 1954. Some aspects of olfactory perception in migrating adult coho and spring salmon. J. Fish Res. Bd. Canada 11: 310–318.

HARA, T. J. 1970. An electrophysiological basis for olfactory discrimination in homing salmon: a review. J. Fish. Res. Bd. Canada 27: 565–586.

1971. Chemoreception, p. 79–120. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 5. Academic Press, Inc., New York, N.Y.

1972. Olfactory responses to amino acids in rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. (in press)

HASLER, A. D. 1966. Underwater guideposts — homing of salmon. Univ. of Wisconsin Press, Madison, Wis. 155 p.

HIDAKA, I. 1970. The effects of transition metals on the palatal chemoreceptors of the carp. Jap. J. Physiol. 20: 599–609.

HIDAKA, I., AND S. YOKOTA. 1967. Taste receptor stimulation by sweet-tasting substances in the carp. Jap. J. Physiol. 17: 652–666.

IDLER, D. R., U. H. FAGERLUND, AND H. MAYOH. 1956. Olfactory perception in migrating salmon. I. L-Serine, a salmon repellent in mammalian skin. J. Gen. Physiol. 39: 889–892.

MCBRIDE, J. F., D. R. IDLER, R. E. E. JONAS, AND N. TOMLINSON. 1962. Olfactory perception in juvenile salmon. I. Observations on response of juvenile sockeye to extracts of foods. J. Fish. Res. Bd. Canada 19: 327–334.

SUTTERLIN, A. M., AND N. SUTTERLIN. 1970. Taste responses in Atlantic salmon (*Salmo salar*) parr. J. Fish. Res. Bd. Canada 27: 1927–1942.

1971. Electrical responses of the olfactory epithelium of Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 28: 565–572.

SUZUKI, N., AND D. TUCKER. 1971. Amino acids as olfactory stimuli in freshwater catfish, *Ictalurus catus* (Linn.). Comp. Biochem. Physiol. 40: 399–404.

WRIGHT, R. H. 1964. The science of smell. George Allen and Unwin Ltd., London, England. 164 p.

YUR'EVA, G. Y. 1957. The role of reactive groups in protein complexes in taste receptor stimulation. Biophysics 2: 653–656.

# Effect of Shelters on the Resistance of Dominant and Submissive Bluegills (*Lepomis macrochirus*) to a Lethal Concentration of Zinc

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SPARKS, R. E., W. T. WALLER, AND J. CAIRNS, JR. 1972. Effect of shelters on the resistance of dominant and submissive bluegills (*Lepomis macrochirus*) to a lethal concentration of zinc. J. Fish. Res. Bd. Canada 29: 1356–1358.

When pairs of bluegills (*Lepomis macrochirus* Rafinesque) were kept in bare, 10-gal compartments for 5 days, dominant–submissive relations developed, and the dominant fish survived a subsequent exposure to 32 mg/liter zinc longer than submissive fish. A flowerpot shelter in each compartment reduced the number of aggressive encounters between fish and removed the response difference. These results indicate that dominant–submissive relations may be a source of variability in the results of bioassays that use fish.

SPARKS, R. E., W. T. WALLER, AND J. CAIRNS, JR. 1972. Effect of shelters on the resistance of dominant and submissive bluegills (*Lepomis macrochirus*) to a lethal concentration of zinc. J. Fish. Res. Bd. Canada 29: 1356–1358.

Lorsque des crapets à oreilles bleues (*Lepomis macrochirus* Rafinesque) sont gardés en paires dans des compartiments de 10 gallons sans abri pendant cinq jours, il s'établit entre les deux un rapport dominance–soumission. Par la suite, les poissons dominants survivent plus longtemps que les poissons soumis, lorsque exposés à des teneurs en Zn de 32 mg/litre. Un pot de fleur formant abri placé dans chaque compartiment réduit les attaques et élimine les réponses différentielles des poissons. Ces résultats suggèrent que les rapports dominance–soumission peuvent être une source de variation dans les essais biologiques qui utilisent les poissons.

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ONE major problem associated with bioassays that utilize fish as test organisms is variation in response among fish. The bluegill (*Lepomis macrochirus* Rafinesque) is one of the warmwater species suggested for use in bioassays by the U.S. Environmental Protection Agency. McDonald and Heimstra (1965) have shown that bluegills are very aggressive and set up dominant–submissive relations when confined in aquaria. This study was undertaken to determine whether dominant and submissive bluegills would have different times to death when exposed to a lethal concentration of zinc and whether shelters would affect survival times.

*Materials and methods* — Bluegill sunfish were seined from a local pond and held for several months in 50-gal stock tanks. The photoperiod in the stock room was 12½ hr of light followed by 11½ hr of darkness. Vita-Lite fluorescent bulbs[3] were used to approximate the spectrum of natural light and a dimming rheostat simulated a 30-min "dawn" starting at 6:30 AM and a 30-min "dusk" starting at 6:30 PM. The water temperature was 22 ± 2 C. Experiments were run in the stock room, under the same light and temperature regimes.

Bluegills ranging in total length from approximately 12 to 16 cm were taken from the stock tanks, roughly matched for length in pairs, and marked by clipping a notch in the soft dorsal fin of one fish and the soft anal fin of the other fish. Each pair was placed in a 10-gal compartment made by dividing a 20-gal aquarium in half with an opaque, perforated tank divider.[4] A continuous flow of dechlorinated municipal tapwater entered one compartment, flowed through the divider into the other compartment, and out of the aquarium through a plastic standpipe. Each pair of fish was fed four ½-g lumps of Gordon Formula fish food (Axelrod 1952) daily.

In replicate experiments 1 and 2, one standard clay flowerpot (upper rim-to-rim diameter: 6 inches) was

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placed on its side as a shelter in each compartment. No pots were used in replicate experiments 3 and 4. Except for the presence or absence of pots, all conditions were the same in the four experiments.

Eleven dominant–submissive pairs were used in each time-to-death bioassay. The test containers were 5-gal glass jars. Ten minutes prior to the addition of fish, a test solution containing 32 ppm $Zn^{++}$ was prepared, using dechlorinated tapwater and Reagent Grade $ZnSO_4 \cdot 7H_2O$ (Fisher catalog no. Z-68). Twenty jars were each filled with 17 liters of test solution, and two control jars were filled with dechlorinated tapwater. The water in every jar was continuously aerated during the experiment. The fish were each placed in separate jars during a 10-min period.

The fish were checked every hour around the clock, and dead fish were immediately removed, weighed, and measured. The criterion for death was absence of response to prodding with a glass rod and cessation of gill movements. The gonads of all fish were examined, but it was possible to determine the sex of both individuals in only nine of the 40 pairs exposed to zinc.

A Kolmogorov-Smirnov test for normality and an analysis of variance were performed on the weights, standard lengths, and times-to-death of fish exposed to zinc. Based on these analyses, data from replicate experiments were combined except for the times-to-death in experiments 3 and 4. In experiment 4 the submissive fish in pair 3 had the second-longest survival time (33 hr) of all the fish exposed to zinc, while the survival times of the other submissive fish in the same experiment ranged from 5 to 8 hr (Table 1). As a result of this aberrant response, the survival times in experiment 4 were not normally distributed and the variance was heterogeneous with respect to the variance in experiment 3, so the survival times could not be pooled. The survival times in experiment 4 were analyzed by Wilcoxon's signed-ranks test, which does not assume normality.

Although fish had been roughly matched for length when they were paired, the dominant fish were significantly longer ($\alpha < .05$) and heavier ($\alpha < .005$) than the submissive fish in experiments with flowerpot shelters. In experiments with no shelters the differences were not significant ($\alpha > .05$).

All control fish (one dominant and one submissive fish exposed to dilution water alone in each of four experiments) survived the 96-hr duration of the bioassays.

*Results and discussion* — When pairs of bluegills established dominant–submissive relations in bare compartments, the dominant fish survived significantly longer in subsequent time-to-death bioassays than submissive fish (experiments 3 and 4, Table 1); with flowerpot shelters in the compartments the difference in response was removed. The difference in survival times of dominant and submissive fish can probably be attributed to stress (as defined by Selye 1955) placed on submissive fish by the attacks of dominant fish. The shelters probably reduced the response differences by reducing encounters, and hence the stress on the submissive fish. Christian (1961), Christian and Davis (1964), and Thiessen (1964) have reviewed literature indicating that dominant individuals in a population are not only under less stress but also seem able to stand more stress (such as disease) than subordinate animals. Pharmacologists recognize that grouping of animals increases the toxicity of many drugs (e.g. Greenblatt and Osterberg 1961).

TABLE 1. Times-to-death (*hours*) of dominant and submissive fish exposed to 32 mg/liter zinc after being paired in tanks with and without flowerpot shelters.

| Experiment | | Pair No. 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean | Range |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *with shelters* | | | | | | | | | | | | | |
| 1 | Dom | 11 | 33 | 18 | 12 | 20 | 12 | 13 | 9 | 21 | 16 | 16.5 | 9–33 |
| | Sub | 11 | 29 | 5 | 6 | 11 | 14 | 12 | 10 | 26 | 8 | 13.2 | 5–29 |
| 2 | Dom | 12 | 9 | 8 | 11 | 10 | 9 | 10 | 11 | 10 | 16 | 10.6 | 8–16 |
| | Sub | 9 | 10 | 11 | 17 | 15 | 11 | 7 | 6 | 9 | 7 | 10.2 | 6–17 |
| *without shelters* | | | | | | | | | | | | | |
| 3 | Dom | 17 | 19 | 15 | 14 | 9 | 12 | 11 | 14 | 15 | 13 | 13.9[a] | 9–19 |
| | Sub | 10 | 9 | 12 | 6 | 6 | 9 | 7 | 9 | 6 | 16 | 9.0[a] | 6–16 |
| 4 | Dom | 20 | 37 | 12 | 29 | 21 | 14 | 18 | 15 | 27 | 9 | 20.2[b] | 9–37 |
| | Sub | 5 | 5 | 33 | 7 | 6 | 9 | 8 | 7 | 7 | 8 | 9.5[b] | 5–33 |

[a]Difference significant at $\alpha < .005$, analysis of variance.
[b]Difference significant at $\alpha = .02$, Wilcoxon's signed-ranks test for paired observations.

It is unlikely that the difference in survival of dominant and submissive fish in our experiments was due to sex or size differences. Of nine pairs of fish in our experiment where both individuals were sexed, six were composed of females, two of males, and one of a dominant male and a submissive female. Hale (1956), working with pairs of green sunfish (*Lepomis cyanellus* Rafinesque) composed of the three possible combinations of two sexes, observed no sex differences in level of aggressiveness or dominance. In our experiments, there was no significant difference in length ($\alpha > .05$) or weight ($\alpha > .05$) of the dominant and submissive fish maintained in compartments with no shelters.

Although fish normally are not acclimated in pairs in small containers in bioassay laboratories, dominance hierarchies can develop in stock tanks, and our results suggest that variability in bioassays might be reduced if fish were held individually for the 10-day minimal acclimation period recommended by American Public Health Association et al. (1971) prior to use in a bioassay. It is likely that dominant–submissive relations can affect bioassay results when several fish are tested together in the 5-gal jars or small aquaria usually used in bioassays. If it is not possible to hold or test fish individually, an effort should be made not to utilize fish in stock tanks that are obviously dominant or obviously submissive, if variability in response is to be minimal.[5]

[5]Five tables giving the lengths and weights of the fish used in these experiments, the number of pairs which established dominant–submissive relations, and the temperature and chemistry of the test solutions are available from ASIS-National Auxiliary Publications Service, c/o CCM Information Corp., 866 Third Avenue, New York, New York, 10022, U.S.A. Microfiche $2.00. Photocopy $5.00. Document NAPS 01824.

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American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1971. Standard methods for the examination of water and wastewater. 13th ed. Washington, D.C. 874 p.

Axelrod, J. H. R. 1952. Tropical fish as a hobby. George Allen and Unwin, Ltd., London, England. 264 p.

Christian, J. J. 1961. Phenomena associated with population density. Proc. Nat. Acad. Sci. USA 47: 428–449.

Christian, J. J., and D. E. Davis. 1964. Endocrines, behavior and population. Science 146: 1550–1560.

Greenblatt, E. W., and A. C. Osterberg. 1961. Correlations of activating and lethal effects of excitatory drugs in grouped and isolated mice. J. Pharmacol. Exp. Ther. 131: 115–119.

Hale, E. B. 1956. Effects of forebrain lesions on the aggressive behavior of green sunfish, *Lepomis cyanellus*. Physiol. Zool. 29: 107–127.

McDonald, A. L., and N. W. Heimstra. 1965. Agonistic behavior in several species of fish. Psychol. Rep. 16: 845–850.

Selye, H. 1955. Stress and disease. Science 122: 625–631.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman Co., Ltd., Washington, D.C. 776 p.

Thiessen, D. D. 1964. Population density and behavior: a review of theoretical and physiological contributions. Tex. Rep. Biol. Med. 22: 266–314.

# Effect of Two Iodophors on Bacterial and Fungal Fish Pathogens

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In in-vitro tests on nine species of bacteria and two species of fungi, the majority of strains tested did not survive a 5-min exposure to either Betadine or Wescodyne at a concentration of 25 ppm active ingredient.

Ross, A. J., and C. A. Smith. 1972. Effect of two iodophors on bacterial and fungal fish pathogens. J. Fish. Res. Bd. Canada 29: 1359–1361.

Au cours d'essais in vitro sur neuf espèces de bactéries et deux de champignons, la majorité des souches n'ont pu survivre à une exposition de 5 minutes à l'un ou l'autre des désinfectants Bétadine ou Wescodyne à une teneur de 25 ppm d'ingrédient actif.

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McFadden (1969) reported that povidone-iodine (Betadine), an organic iodine compound, was an effective egg disinfectant against *Aeromonas liquefaciens*. His tests consisted in treating rainbow trout that were naturally and artificially exposed to the bacterium. He also demonstrated that the compound was relatively nontoxic to eyed trout eggs.

The present work was undertaken to determine the effects of Betadine and Wescodyne, a related organic iodine compound, against selected bacterial and fungal pathogens of fish.

*Materials and methods* — Cultures tested included four strains each of *Aeromonas salmonicida*, *A. liquefaciens*, *Vibrio anquillarum*, *Cytophaga psychrophila*, *Chondrococcus columnaris*, redmouth bacterium (Ross et al. 1966), fluorescent pseudomonads, and nonfluorescent pseudomonads. Also tested were two strains of a kidney disease bacterium (*Corynebacterium* sp.) and one strain each of the fungi *Phoma herbarum* and *Saprolegnia parasitica*. The organisms were grown for 48 hr on Trypticase Soy Agar (TSA-BBL) unless a special medium was required as in the case of *Cytophaga psychrophila*, *Chondrococcus columnaris*, the kidney disease bacterium, and the fungi. Growth was aseptically removed from the culture medium and added to sterile 0.85% physiological saline. The suspension was mixed for about 1 min to obtain a homogeneous suspension and adjusted to the desired optical density, then cooled to 10 C. Five milliliters of the suspension was used to prepare serial dilutions, from which standard pour plates were made to determine the initial number of organisms present.

Iodine solutions were made at 50 ppm active ingredient in distilled water containing 0.05% $NaHCO_3$ giving a *p*H of 7.0. These were also cooled to 10 C prior to use. The procedure for evaluating the germicidal activity was a modification of the technique described by Hays et al. (1967). Tests were carried out at room temperature. As the temperature of our samples increased about 0.5 C min, the 300-sec exposure was conducted at 10–13 C. Five milliliters of culture suspension was added to 5 ml of the iodine solution. At the end of 15, 30, 60, 120, and 300 sec, 5 ml of the iodine-organism mixture was transferred to a sterile petri dish containing 6 ml of sufficient thiosulfate to neutralize the iodine (6 ml of 0.0004 M sodium thiosulfate). The plate was immediately swirled and 25 ml of cooled, melted agar was added with continued swirling to obtain an even dispersion of cells before counting.

Selected cultures were also tested at *p*H 8.0, substituting Tris buffer for $NaHCO_3$.

*Results and discussion* — Except for one strain of *A. salmonicida* exposed to Betadine and *S. parasitica*, which was totally resistant, there was a great decrease in the number of viable organisms following 15-sec exposure to the iodophores at *p*H 7.0 (Table 1). At the end of a 5-min exposure, three strains of pseudomonads exposed to Betadine were not killed, but gave counts of viable organisms ranging from 1–7 cells/ml of suspension. Because under usual hatchery conditions eggs will not be exposed to or carry as high a bacterial load as used in these tests, we believe that a 5-min dip in

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TABLE 1. Numbers (*per ml*) of various strains of bacteria and fungi surviving exposure to 25 ppm of Betadine and of Wescodyne at *p*H 7.0.

| Culture | Betadine | | | | | | Wescodyne | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. cells exposed (*per ml*) | Exposure time (*sec*) | | | | | No. cells exposed (*per ml*) | Exposure time (*sec*) | | | | |
| | | 15 | 30 | 60 | 120 | 300 | | 15 | 30 | 60 | 120 | 300 |
| *Aeromonas salmonicida* | $1\times10^7$ | 34 | 12 | 4 | 1 | NG | $2.6\times10^7$ | 3 | NG | NG | NG | NG |
| | | 26 | 7 | 6 | NG[a] | NG | | 50 | 4 | 19 | NG | NG |
| | | 22 | 13 | 5 | 2 | NG | | 1 | 7 | 1 | NG | NG |
| | | TNTC[b] | TNTC | TNTC | TNTC | NG | | NG | NG | NG | NG | NG |
| *Aeromonas liquefaciens* | $1\times10^7$ | NG | NG | NG | NG | NG | $5\times10^6$ | 51 | NG | NG | NG | NG |
| | | 2 | 1 | NG | NG | NG | | 2 | 38 | NG | NG | NG |
| | | 8 | 2 | NG | NG | NG | | NG | NG | NG | NG | NG |
| | | 10 | 18 | 4 | NG | NG | | 24 | 14 | 2 | NG | NG |
| Redmouth | $2\times10^7$ | NG in all four strains tested | | | | | $3\times10^7$ | NG in all four strains tested | | | | |
| *Vibrio anquillarum* | $1\times10^5$ | NG in all four strains tested | | | | | $3\times10^7$ | 2 | NG | NG | NG | NG |
| | | | | | | | | 16 | 8 | 1 | NG | NG |
| | | | | | | | | 27 | NG | NG | NG | NG |
| | | | | | | | | 3 | 10 | NG | NG | NG |
| Fluorescent pseudomonads | $3\times10^6$ | 2 | 1 | 3 | NG | NG | $5\times10^6$ | 3 | 5 | 2 | 2 | NG |
| | | 60 | 22 | 11 | 3 | 1 | | 15 | 7 | 1 | NG | NG |
| | | 2 | 4 | 1 | NG | NG | | 12 | 6 | NG | 8 | NG |
| | | 25 | 21 | 17 | NG | 1 | | 8 | 10 | NG | NG | NG |
| Nonfluorescent pseudomonads | $9\times10^6$ | NG | 2 | 1 | NG | 7 | $1\times10^7$ | 44 | 19 | 9 | NG | NG |
| | | 9 | 2 | NG | NG | NG | | 6 | 7 | 1 | NG | NG |
| | | 3 | 12 | NG | NG | NG | | 21 | 14 | 10 | NG | NG |
| | | 6 | 3 | 6 | NG | NG | | 8 | 6 | 1 | 2 | NG |
| *Chondrococcus columnaris* | $2\times10^6$ | NG | NG | NG | 1 | NG | $1.7\times10^6$ | 4 | NG | NG | NG | NG |
| | | NG | NG | NG | 2 | NG | | NG | NG | NG | NG | NG |
| | | NG | NG | NG | NG | NG | | 2 | 5 | NG | NG | NG |
| | | 1 | NG | 1 | NG | NG | | 10 | NG | 1 | 6 | NG |
| *Cytophaga psychrophila* | $1.4\times10^6$ | 5 | NG | NG | NG | NG | $1.4\times10^6$ | NG | NG | NG | NG | NG |
| | | 38 | 2 | NG | 1 | NG | | NG | NG | NG | NG | NG |
| | | NG | NG | NG | NG | NG | | 15 | 3 | NG | NG | NG |
| | | NG | NG | NG | NG | NG | | 1 | NG | NG | NG | NG |
| Kidney disease | growth | NT[c] | NT | NT | NT | NG | growth | NT | NT | NT | NT | NG |
| | growth | NT | NT | NT | NT | NG | growth | NT | NT | NT | NT | NG |
| *Phoma herbarum* (spores) | growth | 49 | 18 | 1 | NG | NG | growth | 15 | 11 | 14 | NG | NG |
| *Saprolegnia parasitica* (mycelium) | growth | G[d] | G | G | G | G | growth | G | G | G | G | G |

[a]NG = no growth.
[b]TNTC = too numerous to count.
[c]NT = not tested.
[d]G = growth.

TABLE 2. Numbers (*per ml*) of single strains of bacteria and fungi surviving exposure to 25 ppm of Betadine (B) and of Wescodyne (W) at *p*H 8.0.

| Culture and initial no. of cells exposed (*per ml*) | | Exposure time (*sec*) | | | | |
|---|---|---|---|---|---|---|
| | | 15 | 30 | 60 | 120 | 300 |
| *Aeromonas salmonicida* ($4\times10^7$) | B | 2 | 8 | 1 | 1 | NG[a] |
| | W | 142 | 4 | NG | 1 | NG |
| *Aeromonas liquefaciens* ($1\times10^6$) | B | 24 | 4 | 11 | 1 | 9 |
| | W | 3 | 2 | 2 | 1 | 3 |
| Fluorescent pseudomonads ($2\times10^6$) | B | 18 | 6 | 18 | NG | NG |
| | W | 41 | 33 | 9 | 7 | NG |
| Nonfluorescent pseudomonads ($2\times10^6$) | B | 4 | NG | NG | NG | NG |
| | W | 25 | 10 | NG | NG | NG |
| Redmouth ($5\times10^6$) | B | NG | NG | NG | NG | NG |
| | W | NG | NG | NG | NG | NG |
| *Vibrio anquillarum* ($5\times10^6$) | B | 28 | 9 | 5 | 2 | NG |
| | W | 22 | 10 | NG | NG | NG |
| *Chondrococcus columnaris* ($4\times10^4$) | B | 13 | 4 | 1 | NG | NG |
| | W | 3 | NG | NG | NG | NG |
| *Cytophaga psychrophila* ($5\times10^3$) | B | 6 | NG | NG | NG | NG |
| | W | NG | NG | NG | NG | NG |
| Kidney disease (growth) | B | NT[b] | NT | NT | NT | NG |
| | W | NT | NT | NT | NT | NG |

[a]NG = no growth.
[b]NT = not tested.

Betadine or Wescodyne at 25 ppm can be used to protect against the bacterial species used in this study. The practical effectiveness of these agents, however, will be governed to some extent by the organic load present in the dip water, the possible interrelation between the bacteria and the egg surface and the *p*H, as pointed out by Amend and Pietsch (1972). These authors showed that as the *p*H rises above 7.0 the iodophores become less effective. Although our tests at *p*H 8.0 (Table 2) utilized a limited number of bacterial strains, the results were similar to those obtained at *p*H 7.0, indicating that small changes in alkalinity do not alter adversely the effectiveness of the iodophores.

AMEND, D. F., AND J. P. PIETSCH. 1972. Virucidal activity of two iodophors to salmonid viruses. J. Fish. Res. Bd. Canada 29: 61–65.

HAYS, H., P. R. ELLIKER, AND W. E. SANDINE. 1967. Microbial destruction by low concentrations of hypochlorite and iodophor germicides in alkaline and acidified water. Appl. Microbiol. 15: 575–581.

MCFADDEN, T. W. 1969. Effective disinfection of trout eggs to prevent egg transmission of *Aeromonas liquefaciens*. J. Fish. Res. Bd. Canada 26: 2311–2318.

ROSS, A. J., R. R. RUCKER, AND W. H. EWING. 1966. Description of a bacterium associated with redmouth disease of rainbow trout (*Salmo gairdneri*). Can. J. Microbiol. 12: 763–770.

# Failure to Observe Changes in Selected ... ites Following Removal of the Stannius Corpuscles ... Freshwater North American Eel, *Angu...a rostrata*

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BUTLER, D. G. 1972. Failure to observe changes in selected metabolites following removal of the Stannius corpuscles from the freshwater North American eel, *Anguilla rostrata*. J. Fish. Res. Bd. Canada 29: 1362–1364.

There were no measurable changes in plasma glucose, protein, or free fatty acid concentrations in the freshwater North American eel, *Anguilla rostrata*, 2 weeks after excision of the Stannius corpuscles. Liver glycogen concentrations were normal but muscle glycogen increased. Plasma Na decreased and K and Ca increased. These data indicate that secretions of the Stannius corpuscles are primarily concerned with ionoregulation.

BUTLER, D. G. 1972. Failure to observe changes in selected metabolites following removal of the Stannius corpuscles from the freshwater North American eel, *Anguilla rostrata*. J. Fish. Res. Bd. Canada 29: 1362–1364.

La teneur en glucose, en protéines ou en acides gras libres du plasma de l'anguille d'Amérique d'eau douce, *Anguilla rostrata*, n'a pas changé appréciablement deux semaines après excision des corpuscules de Stannius. La teneur en glycogène est demeurée normale dans le foie, mais a augmenté dans le muscle. Le Na plasmatique a diminué, alors que K et Ca ont augmenté. D'après ces résultats, la fonction primaire des corpuscules de Stannius serait l'ionorégulation.

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IN the North American eel *Anguilla rostrata* (LeSueur) the paired corpuscles of Stannius (Stannius 1839) are imbedded in the ventral surface of the posterior kidney. Ablation of these glands is followed by well-documented changes in plasma and muscle ionic concentrations in both European (Chan et al. 1967) and North American eels (Butler 1969). The physiological basis for these changes is not understood. In addition to releasing a substance, possibly a steroid, that directly or indirectly affects ionoregulation, the corpuscles may secrete a physiologically active peptide that influences protein, fat, or carbohydrate metabolism. With this in mind, plasma protein, fatty acid (FFA), and glucose concentrations together with liver and muscle glycogen concentrations were measured in freshwater North American eels, two weeks after removal of the Stannius corpuscles.

*Materials and Methods* — Silver female eels were collected from the St. Lawrence River near Quebec City, Que., in November 1969. They were taken to the Department of Zoology and held in tanks supplied with dechlorinated tap water (5 C) until April 1970. Twenty-one eels were selected from stock and acclimated to a temperature of 16 C for 2 weeks before the experiments were started. At no time were the eels fed.

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*Experimental groups* — Twenty-one eels were divided into three groups of seven. Group 1 served as intact controls. Eels in group 2 were treated in approximately the same way as those in group 3, except that the Stannius corpuscles were not removed. Eels in group 3 were anesthetized in an aqueous solution of methane tricaine-sulphonate (0.4 g/liter) and the Stannius corpuscles were removed via a 2-inch lateral incision at the level of the posterior kidney. The incision was closed with stainless steel wire. There was no apparent damage to the urinary bladder or ureters. Details of the procedure are given by Butler (1969). Sham-operated and Stanniectomized eels were returned to the holding tanks for 2 weeks, then sampled.

*Sampling procedure* — The sampling procedure was similar for all experimental animals. Each eel was carefully removed from the holding tank, wrapped in wet towelling, and turned on its back. The eel would generally lie motionless while a 5–6-ml blood sample was withdrawn from the caudal vein. The blood sample was centrifuged at 3000 rpm (0 C) for 10 min; plasma was decanted and stored in sealed test tubes at –20 C.

After the blood sample had been collected, the eel was decapitated with a hand axe. Liver and epaxial muscle samples were quickly excised, transferred to a mortar containing liquid nitrogen, and ground to a fine powder. Approximately 1 g of frozen ground liver or muscle was weighed and transferred to a known volume of hot (100 C), 30% aqueous KOH solution and digested for 2 hr. The KOH digests, diluted to a given volume with

distilled water, were stored at room temperature and later used for glycogen assays.

*Assay procedures* — Plasma protein was quantified using the biuret reaction (Gornall et al. 1949); glucose using a glucose oxidase method (Washka and Rice 1961). Plasma free fatty acids (FFA) were extracted and assayed according to the method of Trout et al. (1960). Liver and muscle glycogen concentrations were measured in KOH digestates using the method of Carroll et al. (1956) as modified by Butler (1968). Plasma Na and K concentrations were measured in suitably diluted samples with a Zeiss PF 5 flame photometer; Cl amperimetrically with a Buchler-Cotlove chloridometer. Plasma Ca concentrations were measured by flame emission spectroscopy at 422.7 mμ with a Zeiss PMQ 11 spectrophotometer equipped with a double monochromator and flame attachment.

*Results and Discussion* — Table 1 shows that removal of the corpuscles of Stannius was followed by a statistically significant reduction in plasma sodium concentrations together with significant increases in both potassium and calcium. These changes are typical of those observed in Stanniectomized European (Fontaine 1964; Leloup-Hatey 1964; Fontaine 1967; Chan et al. 1967; Chan 1969) and North American eels (Butler 1969) and, in the present experiments, they serve as indirect evidence that the corpuscles had actually been removed.

Attempts to show that the Stannius corpuscles are either the site of biosynthesis or storage of a steroid with mineralocorticoid properties have been largely unsuccessful (Ford 1959; Phillips and Mulrow 1959; Fontaine and Leloup-Hatey 1959; Idler and Freeman 1966; Sandor et al. 1966). Moreover, Chieffi and Botte (1963) and Hanke and Chester Jones (1965) have failed to demonstrate histochemically the presence of 3β hydroxysteroid dehydrogenase in Stannius corpuscles of the same species. This enzyme is essential for steroidogenesis. These findings are not surprising when one realizes that the corpuscles generally originate as outgrowths of the teleost pronephric duct (De Smet 1962; Garrett 1942) and that the fine structure of the composite cells is reminiscent, not of adrenocortical (interrenal) cells, but of cells that secrete protein — for example, the cells of the exocrine pancreas (Palade 1956). The cytoplasm of these cells is richly endowed with round, osmiophilic secretory granules, each surrounded by a limiting membrane together with a well-developed, rough endoplasmic reticulum (Oguri 1966; Fujita and Honma 1967). For these reasons I considered the possibility that one or more proteins, secreted by these endocrine organs, might influence protein, fat, or carbohydrate metabolism. Data in Table 1 show that ablation of the Stannius corpuscles failed to alter significantly the plasma glucose, protein, or FFA concentrations. Plasma glucose concentrations increased in both sham-operated and Stanniectomized eels compared with intact controls, a change that may have been induced by the stress of surgery. Liver glycogen concentrations were unaffected by Stanniectomy, but there was a significant increase in muscle glycogen. The reason for this change remains obscure. The results indicate that a renewed investigation of the influence of the Stannius corpuscles on ionoregulation would be the most profitable area for further research.

BUTLER, D. G. 1968. Hormonal control of gluconeogenesis in the North American eel, *Anguilla rostrata*. Gen. Comp. Endocrinol. 10: 85–91.

1969. Corpuscles of Stannius and renal physiology in the eel (*Anguilla rostrata*). J. Fish. Res. Bd. Canada 26: 639–654.

TABLE 1. Effect of Stanniectomy on the composition of plasma, liver, and muscle from freshwater North American eels (values are means ± SE).

| | | Plasma | | | | | | | Liver | Muscle |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Na (*meq/liter*) | K (*meq/liter*) | Cl (*meq/liter*) | Ca (*meq/liter*) | Glucose (*mg%*) | Protein (*mg%*) | FFA (*μm/ml*) | glycogen (*mg%*) | |
| Controls | 7 | 152.3 ±2.8 | 2.70 ±0.19 | 82.8 ±4.3 | 3.89 ±0.10 | 41.4 ±6.0 | 5.45 ±0.16 | – | 6.59 ±0.79 | 0.165 ±0.019 |
| Sham-operated | 7 | 136.7 ±5.2 | 3.09 ±0.31 | 60.7 ±7.1 | 3.83 ±0.12 | 67.0 ±15.0 | 5.20 ±0.31 | 1.58 ±0.15 | 6.46 ±0.60 | 0.093 ±0.027 |
| Stanniectomized | 6 | 115.8[a] ±6.2 | 5.04[a] ±0.46 | 65.6 ±10.4 | 9.52[a] ±0.98 | 57.7 ±7.3 | 4.77 ±0.13 | 1.52 ±0.18 | 7.19 ±0.87 | 0.304[a] ±0.036 |

[a] $P < 0.05$ compared with sham-operated controls using Duncan's multiple range test.

CARROLL, N. V., R. W. LANGLEY, AND J. H. ROE. 1956. The determination of glycogen in liver and muscle by use of anthrone reagent. J. Biol. Chem. 220: 583–593.

CHAN, D. K. O. 1969. Endocrine regulation of calcium and inorganic phosphate balance in the freshwater-adapted teleost fish, *Anguilla anguilla* and *A. japonica*. Proc. 3rd Int. Congr. Endocrinol., Mexico City, 1968. Excerpta Med. Int. Congr. Ser. No. 184: 709–716.

CHAN, D. K. O., I. CHESTER JONES, I. W. HENDERSON, AND J. C. RANKIN. 1967. Studies on the experimental alteration of water and electrolyte composition of the eel (*Anguilla anguilla* L.). J. Endocrinol. 37: 297–317.

CHIEFFI, G., AND V. BOTTE. 1963. Histochemical reaction for steroid-3$\beta$-ol-dehydrogenase in the interrenal and the corpuscles of Stannius of *Anguilla anguilla* and *Conger conger*. Nature (London) 200: 793–794.

DE SMET, D. 1962. Considerations of the Stannius corpuscles and interrenal tissues of bony fishes, especially based on researches into *Amia*. Acta Zool. (Stockholm) 43: 201–219.

FONTAINE, M. 1964. Corpuscules de Stannius et régulation ionique (Ca, K et Na) du milieu intérieur de l'Anguille (*Anguilla anguilla* L.). C. R. Acad. Sci. (Paris) 259: 875–878.

1967. Intervention des corpuscules de Stannius dans l'équilibre phosphocalcique du milieu intérieur d'un poisson téléostéen, l'anguille (*Anguilla anguilla* L.) C. R. Acad. Sci. (Paris) 264: 736–737.

FONTAINE, M., AND J. LELOUP-HATEY. 1959. Mise en évidence de corticostéroides dans l'interrénal d'un Téléostéen: le Saumon (*Salmo salar* L.). J. Physiol. (Paris) 51: 468–469.

FORD, P. 1959. Some observations on the corpuscles of Stannius, p. 728–734. *In* A. Gorbman [ed.] Comparative endocrinology. John Wiley & Sons, Inc., New York, N.Y.

FUJITA, H., AND Y. HONMA. 1967. On the fine structure of corpuscles of Stannius of the eel, *Anguilla japonica*. Z. Zellforsch. Mikroskop. Anat. 77: 175–187.

GARRETT, F. D. 1942. The development and phylogeny of the corpuscles of Stannius in ganoid and teleostean fishes. J. Morphol. 70: 41–68.

GORNALL, A. G., C. S. BARDAWILL, AND M. M. DAVID. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751–766.

HANKE, L. W., AND I. CHESTER JONES. 1965. Histological and histochemical studies on the adrenal cortex and the corpuscles of Stannius of the European eel, *Anguilla anguilla* L. Gen. Comp. Endocrinol. 5: 683.

IDLER, D. R., AND H. C. FREEMAN. 1966. Steroid transformations by corpuscles of Stannius of the Atlantic cod (*Gadus morhua* L.). J. Fish. Res. Bd. Canada 23: 1249–1255.

LELOUP-HATEY, J. 1964. Modifications de l'équilibre minéral de l'Anguille (*Anguilla anguilla* L.) consécutives à l'ablation des corpuscles de Stannius. C. R. Sci. Biol. 158: 711–715.

OGURI, M. 1966. Electron-microscopic observations on the corpuscles of Stannius in goldfish. Bull. Jap. Soc. Sci. Fish. 32: 903–908.

PALADE, G. E. 1956. Intracisternal granules in the exocrine cells of the pancreas. J. Biophys. Biochem. Cytol. 2: 417–422.

PHILLIPS, J. G., AND P. J. MULROW. 1959. Failure of the corpuscles of Stannius from winter flounder (*Pseudopleuronectes americanus*) to synthesize adrenocorticosteroids *in vitro*. Nature (London) 184: 558.

SANDOR, T., G. P. VINSON, I. CHESTER JONES, I. W. HENDERSON, AND B. J. WHITEHOUSE. 1966. Biogenesis of corticosteroids in the European eel (*Anguilla anguilla* L.). J. Endocrinol. 34: 105–115.

STANNIUS, H. 1839. Die Nebennieren bei Knochenfischen. Arch. Anat. Physiol. Wiss. Med. 97: 101.

TROUT, D. L., E. H. ESTES, AND S. J. FRIEDBERG. 1960. Titration of free fatty acids of plasma: a study of current methods and a new modification. J. Lipid Res. 1: 199–202.

WASHKA, M. E., AND E. W. RICE. 1961. Determination of glucose by an improved enzymatic procedure. Clin. Chem. 7: 542–545.

# Effect of Smoke-Processing on Muddy Odor and Taste in Rainbow Trout (*Salmo gairdneri*)

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IREDALE, D. G., AND D. RIGBY. 1972. Effect of smoke-processing on muddy odor and taste in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada 29: 1365–1366.

Because of problems with a "muddy" odor and taste occurring in rainbow trout being reared commercially in some small prairie lakes in central Canada, full production potential has not been realized. The possibility of masking the undesirable condition by smoke-processing was investigated and this was found to be a valid method of utilizing the majority of the affected fish.

IREDALE, D. G., AND D. RIGBY. 1972. Effect of smoke-processing on muddy odor and taste in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada 29: 1365–1366.

L'odeur et le goût de vase des truites arc-en-ciel élevées pour le commerce dans les petits lacs des Prairies canadiennes ont empêché cette industrie de réaliser son plein potentiel. Après étude, nous avons constaté que le fumage masque efficacement cette condition peu désirable et permet d'utiliser la majorité des poissons affectés.

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EFFORTS to utilize small prairie lakes in central Canada for the commercial rearing of rainbow trout (*Salmo gairdneri*) (Johnson et al. MS 1970) have been plagued by a so-called "muddy" odor and taste in fish from certain lakes. This is a serious problem in some areas, where a large part of the catch may be rejected because of this unacceptable condition. The following study was undertaken to determine if smoke-processing could significantly mask the presence of muddy odor and taste in rainbow trout.

Random samples of rainbow trout from one lake harvested in October 1970 were evaluated by the Department of Fisheries, Inspection Branch, and rejected because of muddy odor and taste. Fish from this lake were smoked according to the following two methods. *Method A* was a trial process, resulting in a very high smoke concentration; *Method B* was designed to achieve a smoke concentration similar to that present in a conventionally smoked dressed fish.

*Treatments* — Method A: Paired skinless fillets from each of 20 fish were treated as follows: a control fillet from each fish was blast-frozen, sealed individually in moisture/vapor proof film, and held at –30 F (–34.4 C); its paired fillet was immersed in 80% saturated sodium chloride brine for 90 sec at 60 F (15.5 C), and smoke-processed for 35 min at 90 F (32.2 C) and 60% relative humidity. The smoked fillets were blast-frozen, sealed individually, and stored at –30 F (–34.4 C).

Method B: Paired fillets with skins on were treated as follows: a control fillet from each fish was blast-frozen, sealed in film, and held at –30 F. Its paired fillet was immersed for 90 sec in 80% saturated sodium chloride brine at 60 F, smoke-processed for 75 min with exposed flesh sealed with saran wrap, followed by 10 min smoking with the saran removed, for a total time of 85 min at 90 F and 60% relative humidity. The fillets were then blast-frozen, sealed individually, and stored at –30 F. Method B provided both an unsmoked fillet as a control, and a corresponding fillet with a smoke concentration approximating that which would be obtained if the dressed fish had been smoked intact.

*Taste panel evaluation* — The following evaluations were conducted to assess the effect of smoking on muddy odor and taste; six experienced panel members evaluated pairs of fillets from six randomly selected fish treated according to Method *A* (Panel I) and Method B treatments (Panel II). Fillets were thawed, divided into six pieces which were individually wrapped in aluminum foil, coded, and cooked over steam for 10 min. Each panel member received one control and one smoke-treated portion from each fish, and indicated whether muddy odor or taste could be detected in either sample, on a score sheet similar to the example shown here:

*Test #* *Date*:
*Product*: *Rainbow trout.* *Name*:

Listed below are three pairs of code numbers corresponding to the code numbers of samples to be evaluated. One sample of each pair has been smoke-treated, the other has not.
For *each sample* indicate whether you can or cannot detect muddiness in either the *odor* or *taste*. If muddiness

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TABLE 1. Taste panel results.

| Assigned statistical value | | Evaluation description categories | Scores (%) Panel I (36 observations) | Panel II (36) | Panel III (32) |
|---|---|---|---|---|---|
| *Muddy odor* | +1 | Control was muddy, smoked sample was clear[a] | 19.4 | 19.4 | 18.8 |
| | 0 | A, Control was muddy, smoked sample was muddy or, B, Control was clear, smoked sample was clear[b] | 80.6 | 77.8 | 75.0 |
| | −1 | Control was clear, smoked sample was muddy[c] | – | 2.8 | 6.2 |
| *Muddy taste* | +1 | Control was muddy, smoked sample was clear[a] | 33.3 | 44.4 | 43.8 |
| | 0 | A, Control was muddy, smoked sample was muddy or, B, Control was clear, smoked sample was clear[b] | 66.7 | 55.6 | 53.1 |
| | −1 | Control was clear, smoked sample was muddy[c] | – | – | 3.1 |

[a]Muddiness was evident in the control, but not in the corresponding smoke-treated samples, demonstrating that smoking was effective in masking muddy odor or taste.

[b]A, Muddiness was evident in the control and in the corresponding smoke-treated samples, showing that smoking was not effective in masking muddy odor or taste; B, Muddiness was not evident in either the control or the smoke-treated sample, presumably the fish was not muddy to a recognizable degree.

[c]Muddiness was not evident in the control but appeared evident in the corresponding smoke-treated sample. In this case the taster was probably confusing some other taste characteristic attributable to the smoke process as muddiness.

appears evident in both samples in a pair, please indicate in which sample the muddiness is more evident.

| *Sample Number* | *Muddy Odor: Yes or No* | *Muddy Taste: Yes or No* | *Sample in which the muddiness is more evident* |
|---|---|---|---|
| *Pair 1* | — — — — | — — — — | — — — — |
| *Pair 2* | — — — — | — — — — | — — — — |
| *Pair 3* | — — — — | — — — — | — — — — |

Panel III, consisted of 32 randomly chosen inexperienced tasters, who were required to evaluate only one pair of samples. The samples that they evaluated were prepared as follows: six pairs of control and smoked fillets treated by Method B were thawed, skins were removed, fillets were minced and blended into control or smoke-treated lots. Individual samples from each lot were wrapped in aluminum foil, coded, cooked over steam for 10 min, and served to the panelists, who were asked whether they could detect any objectionable odor or taste in either sample. They were not asked to state whether they detected "muddiness," since their familiarity with this particular odor and taste was considered questionable. The word "muddy" was therefore changed to "objectionable," and "muddiness" to "objectionable odor" or "objectionable taste" in the score sheets that Panel III used.

*Taste panel results* — Values of (+1), (0), and (−1) were assigned to the taste and odor assessments (Table 1). Mean values were calculated and tested using a simple *t*-test, with the following results:

| | Muddy Odor | Muddy Taste |
|---|---|---|
| Panel I (experienced tasters) | highly significant ($P<0.01$) | highly significant ($P<0.01$) |
| Panel II (experienced tasters) | significant ($P<0.05$) | highly significant ($P<0.01$) |
| Panel III (consumer) (inexperienced tasters) | not significant | highly significant ($P<0.01$) |

The results indicate that muddiness is significantly masked by smoke processing and the effectiveness of masking appears to be related to the intensity of smokiness. This is evidenced by the effectiveness of masking obtained for both muddy odor and taste for panel I, which rated samples wherein the smoke intensity was greater than that of the fish evaluated by panels II and III. The results shown for panels II and III indicate that smoking was significantly effective in masking muddy taste but less effective in masking muddy odor.

JOHNSON, L., G. H. LAWLER, AND L. A. SUNDE. MS 1970. Rainbow trout farming in central Canada. Fish. Res. Board Can. Tech. Rep. 165: 9 p.

# Cadmium Uptake by Marine Organisms

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EISLER, R., G. E. ZAROOGIAN, AND R. J. HENNEKEY. 1972. Cadmium uptake by marine organisms. J. Fish. Res. Bd. Canada 29: 1367–1369.

Adults of mummichog, *Fundulus heteroclitus*, scallop *Aquipecten irradians*, oyster *Crassostrea virginica*, and subadult lobsters *Homarus americanus* were immersed for 21 days in flowing sea water containing 10 μg/liter of cadmium as $CdCl_2 \cdot 2\frac{1}{2}H_2O$. Cadmium residues in whole animals and selected tissues were consistently higher in exposed organisms than controls; edible portions of treated lobster (muscle), scallop (adductor muscle), and oyster (whole animal) contained more cadmium per unit wet weight than controls by 25%, 19%, and 352%, respectively.

EISLER, R., G. E. ZAROOGIAN, AND R. J. HENNEKEY. 1972. Cadmium uptake by marine organisms. J. Fish. Res. Bd. Canada 29: 1367–1369.

Nous avons plongé des choquemorts (*Fundulus heteroclitus*), des pétoncles (*Aquipecten irradians*), des huîtres (*Crassostrea virginica*), tous adultes, et des homards (*Homarus americanus*) presque adultes dans de l'eau de mer à flot continu contentant 10 μg/litre de cadmium sous forme de $CdCl_2 \cdot 2\frac{1}{2}H_2O$ durant 21 jours. Les résidus de Cd dans l'animal entier et dans certains tissus ont été uniformément plus élevés chez les organismes exposés que chez les témoins; les portions comestibles de homard (muscle), de pétoncle (muscle adducteur) et d'huître (animal entier) traités contenaient plus de Cd par unité de poids humide que les témoins, dans des proportions de 25%, 19% et 352% respectivement.

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CADMIUM in drinking water at concentrations above 10 μg/liter (10 ppb) and in food at levels exceeding 13 mg/kg wet weight (13 ppm) is considered hazardous to public health (Anon. 1962, 1963). Cadmium salts from industrial and other sources are found in wastes discharged into estuarine and marine waters (McKee and Wolf 1963). Also, seafoods constitute a major source of cadmium in the human diet (McKee and Wolf 1963). This, and the fact that cadmium at ambient concentrations between 80 and 180 ppb is fatal to at least 25% of three species of marine decapod crustaceans in 96 hr (Eisler 1971) have motivated a series of investigations by our laboratory on accumulation of stable cadmium by marine organisms from sea water containing appreciable levels of cadmium. Thisa ccount reports on cadmium residues in selected tissues from four species of marine organisms, including edible meats from oyster, scallop, and lobster, after immersion for 21 days in seawater solutions containing approximately 10 ppb of $Cd^{2+}$.

*Materials and methods* — Test organisms were subadults of the American lobster, *Homarus americanus*, and adults of the American oyster, *Crassostrea virginica*, the bay scallop, *Aquipecten irradians*, and the mummichog, *Fundulus heteroclitus*, an estuarine cyprinodontiform teleost. All species were collected from Rhode Island waters in the summer of 1971: lobsters of mean length 169 mm from eye to uropod and 160–196 g wet weight from Narragansett Bay were captured by local fishermen; oysters of mean height 51 mm and 4.9–5.1 g wet weight (meats) from the Pawcatuck River; scallops of 49 mm mean height and 6.8–7.7 g wet weight (meats) from Weekapaug Pond; mummichog averaging 62 mm total length and 2.3–2.4 g wet weight from East Matunuck. After capture, all organisms were transported to a floating laboratory located in Jerusalem, R. I., separated by species, and held in U-shaped fiberglass troughs for 28 days. The troughs measured 2.5 m long × 30 cm wide × 25 cm deep and were supplied with flowing, unfiltered sea water of 34‰ salinity and 18.4 C (16–20 C) at the rate of 16 liters/min. During acclimatization and exposure, the lobsters were fed chopped fish twice weekly and mumichog were fed chopped clams three times weekly. Scallops and oysters were not fed because the flowing sea water contained food organisms suitable for filter feeders. Mortality was negligible for all species during the entire study.

After the 28-day holding period, each species was divided into two groups and placed in separate troughs; individual troughs contained either 100 oysters, 100

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scallops, 20 lobsters, or 125 mummichogs. One trough of each species received a cadmium solution ($CdCl_2 \cdot 2\frac{1}{2}H_2O$) and one of each served as controls. The seawater-cadmium solution contained 0.016 mg/ml of $Cd^{2+}$ and was pumped into the troughs at a rate of 10 ml/min, which when mixed with incoming sea water (16 liters/min) produced a mean concentration of 10 ppb of $Cd^{2+}$. A level of 1.0 ppb of $Cd^{2+}$ in the control water was determined by polarography (MASA Model 2014, Environmental Sciences Assoc., Inc.); levels of cadmium in the experimental troughs were checked twice weekly by Dr E. W. Davey (NMWQL) and ranged between 10.8 and 11.2 ppb.

This study, along with many others, was summarily terminated on the 21st day of exposure due to a need for repairs to the hull of the floating laboratory. At that time we collected at least five animals (maximum 17) from each trough and prepared these for cadmium analysis by atomic absorption spectroscopy, using the procedures of Eisler and LaRoche (1972).

A total of 16 pooled tissue samples were prepared, 8 experimental and 8 control. Samples were (1) whole mummichog, (2) whole oyster meats, (3) scallop adductor muscle, (4) scallop remaining soft parts, (5) lobster muscle from abdomen and claws, (6) lobster gill, (7) lobster viscera, and (8) lobster remainder, which was comprised almost entirely of exoskeleton. The sample preparation procedures, originally designed for whole killifish, involve dry-ashing and digestion in 6N HCl. In the present study, undigested materials in mummichog samples comprised less than 5%, though this amount was higher in samples from the other three species. Cadmium added as an internal standard at increments of 0.5 ppm was recovered at 98–100%, provided that the minimum cadmium level was 4 mg/kg ash. Results were expressed in milligrams Cd/kilogram ash and also as milligrams Cd/kilogram wet weight on the basis of wet weight/ash ratios determined for individual samples.

*Results and discussion* — Cadmium levels in pooled samples from treated organisms were consistently higher than controls (Table 1). This clearly demonstrates that all species tested accumulated measurable amounts of cadmium in 3 weeks from sea water that, from a public health viewpoint, contained theoretically safe levels of $Cd^{2+}$. The U.S. Public Health Service (Anon. 1962) emphasizes the fact that it is the *concentration* of cadmium in water and food and not the *absolute amount* that determines acute cadmium toxicity. Whole American oysters, on this basis, quickly accumulate emetic threshold (ET) concentrations of cadmium, viz 13–15 ppm wet weight, when $Cd^{2+}$ levels in flowing sea water exceed 10 ppb; ET levels were surpassed in 5 weeks and 2 weeks at $Cd^{2+}$ levels of 25 and 100 ppb, respectively (Shuster and Pringle 1969). Because our data were based on a single analysis of tissues after one exposure period only, it is impossible at this time to predict whether oysters or other species that we tested can accumulate ET levels of cadmium at ambient concentrations of 10 ppb or lower of $Cd^{2+}$. Additional research is also required to determine if higher trophic levels directly in man's food chain can accumulate ET concentrations of cadmium from mummichogs and other forage species. Nevertheless, one fact remains: seafoods that are normally ingested directly by man,

TABLE 1. Cadmium in whole body and selected tissues of four marine species after immersion for 21 days in flowing sea water containing 10 ppb of cadmium (as $CdCl_2 \cdot 2\frac{1}{2}H_2O$) (values in parenthesis represent percentage increases over controls).

| Species | mg Cd/kg ash wt | | mg Cd/kg wet wt | |
|---|---|---|---|---|
| | Experimental | Control | Experimental | Control |
| *Crassostrea virginica* | | | | |
| whole oyster | 52.1(460) | 9.3 | 1.49(352) | 0.33 |
| *Aquipecten irradians* | | | | |
| whole scallop[a] | 82.7(143) | 43.0 | 2.46(114) | 1.15 |
| adductor muscle | 74.1(51) | 49.2 | 1.68(19) | 1.41 |
| remainder | 85.0(188) | 29.5 | 2.74(161) | 1.05 |
| *Homarus americanus* | | | | |
| whole lobster[a] | 5.4(2) | 5.3 | 0.72(41) | 0.51 |
| muscle | 11.7(17) | 10.0 | 0.25(25) | 0.20 |
| exoskeleton | 4.4(7) | 4.1 | 0.88(49) | 0.59 |
| gill | 31.3(82) | 17.2 | 0.87(78) | 0.49 |
| viscera | 37.5(11) | 33.8 | 1.21(0) | 1.21 |
| *Fundulus heteroclitus* | | | | |
| whole fish | 7.6(36) | 5.6 | 0.48(45) | 0.33 |

[a]Estimated from sum of components.

including scallop adductor muscle, lobster meats, and whole oysters, exhibit measurable uptakes of cadmium after immersion of the animals in water containing Cd concentrations not previously considered hazardous to aquatic life or to public health.

ANON. 1962. Drinking water standards. U.S. Dep. Health Educ. Welfare, Pub. Health Serv. Publ. 956: 61 p.

1963. International standards for drinking water. 2nd ed. World Health Organization, Geneva, Switzerland. 206 p.

EISLER, R. 1971. Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. J. Fish. Res. Bd. Canada 28: 1225–1234.

EISLER, R., AND G. LAROCHE. 1972. Elemental composition of the estuarine teleost *Fundulus heteroclitus* (L.). J. Exp. Mar. Biol. Ecol. 8: 201–214.

MCKEE, J. E., AND H. W. WOLF. 1963. Water quality criteria. 2nd ed. Calif. State Water Qual. Contr. Board Publ. 3-A: 548 p.

SHUSTER, C. N., JR., AND B. H. PRINGLE. 1969. Trace metal accumulation by the American eastern oyster, *Crassostrea virginica*. Proc. Nat. Shellfish. Ass. 59: 91–103.

## Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

(1) Fisheries Research Board of Canada
Biological Station
P.O. Drawer 100
Nanaimo, British Columbia

(2) Fisheries Research Board of Canada
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver 8, British Columbia

(3) Fisheries Research Board of Canada
Pacific Environment Institute
4160 Marine Drive
West Vancouver, British Columbia

(4) Fisheries Research Board of Canada
Freshwater Institute
501 University Crescent
Winnipeg 19, Manitoba

(5) Fisheries Research Board of Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec

(6) Fisheries Research Board of Canada
Biological Station
St. Andrews, New Brunswick

(7) Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia

(8) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(9) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

1677. BERKELEY, C. 1972. Further records of Polychaeta new to British Columbia with comments on some others. Can. J. Zool. 50: 451–456. (1)

1678. FLETCHER, G. L., AND R. F. ADDISON. 1972. Some aspects of the chemistry and acute toxicity of the iron ore flotation agent bimethyl ammonium alkyl hydroxamate and some related compounds to brook trout. Bull. Environ. Contam. Toxicol. 7: 147–159. (7)

1679. IDLER, D. R., AND P. WISEMAN. 1971. Sterols of molluscs. Int. J. Biochem. 2: 516–528. (8)

1680. BRETT, J. R. 1972. The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Resp. Physiol. 14: 151–170. (1)

1681. MARGOLIS, L., AND M. D. DAILEY. 1972. Revised annotated list of parasites from sea mammals caught off the west coast of North America. Nat. Oceanic Atmos. Admin. Tech. Rep. SSRF-647: 23 p. (1)

1682. HIROSE, K., AND E. M. DONALDSON. 1972. Biological study on ovulation *in vitro* of fish—III. The induction of *in vitro* ovulation of *Oryzias latipes* oocytes using salmon pituitary gonadotropin. Bull. Jap. Soc. Sci. Fish. 38: 97–100. (2)

1683. ARMSTRONG, F. A. J. 1972. Phosphorus compounds in natural waters. Int. Symp. Identification Meas. Environ. Pollutants, Ottawa, Ont. June, 1971: 370–374. (4)

1684. UTHE, J. F., J. REINKE, AND H. GESSER. 1972. Extraction of organochlorine pesticides from water by porous polyurethane coated with selective absorbent. Environ. Lett. 3: 117–135. (4)

1685. UTHE, J. F. 1972. Determination of total and organic mercury levels in fish tissue. Int. Symp. Identification Meas. Environ. Pollutants, Ottawa, Ont. June, 1971: 207–212. (4)

1686. MACCALLUM, W. A. 1971. Quality aspects of industrial water thawing and fillet processing, p. 225–233. *In* R. Kreuzer [ed.] Fish inspection and quality control. Fishing News (Books) Limited, London, England. (9)

1687. WILDISH, D. J. 1972. Post embryonic growth and age in some littoral *Orchestia* (Amphipoda, Talitridae). Crustaceana 3: 267–274. (6)

1688. SWIFT, D. J. P., J. R. SCHUBEL, AND R. E. SHELDON. 1972. Size analysis of fine-grained suspended sediments: a review. J. Sediment. Petrology 42: 122–134. (7)

## Additional FRB Publications

The Fisheries Research Board of Canada also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at Board establishments located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

L'Office des recherches sur les pêcheries du Canada publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements de l'Office situés a Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

Journal
Fisheries
Research
Board
Canada

# Guide to Authors

Details on typing and other items in preparing copy are given in a guide for typists. Also, a list of recommended spellings of about 100 words common in fisheries journals is available from the Office of the Editor.

## Editorial Practices

Abstracts should be provided in both English and French.

The *Style Manual for Biological Journals* (2nd ed. American Institute of Biological Sciences, 2000 P St. NW., Washington, D.C.) is, with few exceptions, used as the authority in matters of form. *Webster's Third New International Dictionary* is the standard for spelling and division of words in English; *le Grand Larousse Encyclopédique*, for those in French. Exceptions are made for manuscripts that follow the *Oxford English Dictionary* entirely. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. Manuscript reports are included in the list of references, but are identified both there and in the text by the letters "MS" before the year. The *Journal* follows the spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

*Notes* are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued before completion; or on preliminary findings that warrant publication before problems are resolved satisfactorily. Or they may be used to establish priority in publication. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

In general, *series titles* should be avoided.

### Illustrations

*Line drawings* are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and legible when the illustration is reduced to the appropriate size. Drawings should be grouped, when feasible, for economy of space. Originals must be submitted and should be less than 20 inches in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Prints of photographs should be made on glossy paper with a wide range in tone. They should be cropped, squared, and mounted with rubber cement on white cardboard, with no space between those arranged in groups.

### Proofs and Reprints

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty reprints, without covers, are supplied free. Additional copies needed must be ordered, on the form provided, when the galley proof is returned.

## Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors. The same questions are recommended to referees who review manuscripts for the *Journal*.

### General

Is there new knowledge in the report worth publishing?

Are the findings, interpretations, and conclusions sound, and relevant to the purpose of the study?

If the manuscript is merely confirmatory, is it worth publishing?

Does the manuscript need to be rewritten before the work can be assessed?

Is the contribution placed in the proper perspective concerning the state of knowledge of the subject?

Is the manuscript too long?

Are all the tables and figures needed, and organized for economy of space?

Would any of the text be clearer if condensed?

Should some of the data be made available separately, in a manuscript report or at a data repository?

Should the manuscript be condensed as a Note?

Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics?

Are there inconsistencies between tables or figures and the text, or within the text?

If the manuscript is from a thesis, does it retain characteristics common in these but not desirable in journals?

### Parts of the Manuscript

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Has the *introduction* all the important guideposts for orienting the reader?

Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data obtained?

Are generalizations on the *results* adequate?

Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

If there are loopholes in interpretation, are they acknowledged? Should they be overcome?

Should other interpretations be recognized?

Journal of the Fisheries Research Board of Canada

# Guide des auteurs

Des détails sur le tapage et certains autres item sont donnés dans le "Guide pour les secrétaires d'auteurs." Aussi, une liste d'épellations recommandées d'environ 100 mots relatifs aux articles sur les pêcheries est disponible au bureau du Directeur de la publication.

## Disposition générale

Les résumés doivent être fournis en anglais et en français.

Le *Style Manual for Biological Journals* (deuxième édition, publié par l'American Institute of Biological Sciences, 2000, rue *P* N.-O., Washington, D.C.), a été adopté comme guide des auteurs. Le *Third New International Dictionary* de Webster, dans le cas de l'anglais, et le *Grand Larousse Encyclopédique*, dans le cas du français, font respectivement autorité pour l'orthographe et la séparation des mots. Une exception est faite à l'égard des travaux en anglais qui se conforment entièrement à l'*Oxford English Dictionary*. Les titres de périodiques sont abrégés selon la *Biological Abstracts*. Toute référence à la littérature doit suivre le système nom-année. Les rapports manuscrits sont inclus dans la liste des références, les lettres "MS" avant l'année les identifient dans les références et dans le texte. Le *Journal* se sert des épellations recommandées dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No 6 de l'American Fisheries Society.

Les *notes* se distinguent des écrits d'après l'étendue de la recherche plutôt que par la qualité de la recherche. Les notes peuvent être sur des travaux de recherche de portée restreinte, susceptibles de découler d'investigations plus considérables, pour la description de techniques de recherche et de méthodes de pêche, pour l'établissement de priorités de publication, et pour faire connaître les renseignements utiles tirés d'entreprises qui ont été interrompues avant d'avoir été achevées. Si la portée de la Note le permet, elle peut être disposée comme un mémoire. D'autre part, la disposition est flexible. La longueur ne doit pas dépasser quatre pages imprimées.

En général, les mémoires en série doivent être évités.

### Les illustrations

Les dessins au trait doivent être exécutés à l'encre de chine sur papier à dessin blanc ou sur papier à calquer, ou sur papier à coordonnées avec quadrillage bleu. Les lignes, les symboles, les lettres et les chiffres doivent tous être propres. Ils doivent être lisibles quand ils sont réduits à la grandeur voulue pour la reproduction désirée. Les dessins doivent être groupés, si la chose est possible, de façon à utiliser tout l'espace disponible. Les originaux doivent être soumis et devraient mesurer moins de 20 pouces dans le sens le plus long pour les illustrations de pages entières, et proportionnellement plus petit pour les illustrations de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves des photographies doivent être tirées sur papier glacé et accuser une gamme étendue de teintes. Elles doivent être coupées, dressées et montées, au moyen de dissolution, sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

### Epreuves et tires a part

Les épreuves en placards sont adressées à l'auteur et la responsabilité de toute erreur qui peut y rester lui incombe.

Cinquante tirés à part, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander d'autres tirés à part.

## Liste de contrôles pour manuscrits

Une attention particulière portée sur les questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs. Les mêmes questions sont recommandées aux arbitres qui font la critique des livres pour le *Journal*.

### Generalites

Est-ce que les connaissances nouvelles du rapport méritent d'être publiées?

Les découvertes, les interprétations et les conclusions sont-elles solides et pertinentes au but de l'étude?

Si le manuscrit est tout simplement corroboratif, mérite-t-il d'être publié?

Le manuscrit doit-il être récrit avant d'évaluer le travail?

La contribution est-elle placée dans une perspective appropriée relative à l'état des connaissances du sujet?

Le manuscrit est-il trop long?

Les tables et figures sont-elles toutes nécessaires et arrangées pour économiser le plus d'espace?

Le texte serait-il plus clair s'il était condensé?

Est-ce qu'une partie des données devrait être disponible séparément, soit dans un rapport ou dans un dépôt des données?

Le manuscrit devrait-il être condensé en une note?

Est-ce que la préparation du manuscrit suit la logique mentionnée dans l'introduction?

Y a-t-il des exposés sommaires au début de chaque section et paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents aux sujets?

Y a-t-il des contradictions entre les tableaux et les figures du texte, ou dans le texte même?

Si le manuscrit est tiré d'une thèse, contient-il des caractéristiques communes à cette dernière, mais indésirables dans un journal?

PARTIES DU MANUSCRIT

Le titre renferme-t-il le contenu du rapport?

Le résumé donne-t-il l'essentiel des connaissances nouvelles?

L'introduction a-t-elle tous les points importants afin d'orienter le lecteur?

Le renvoi bibliographique se limite-t-il à la définition du problème?

Les détails du matériel et des méthodes employées sont-ils limités à un minimum pour que les scientifiques comprennent le but de l'étude et puissent porter un jugement adéquat sur les données obtenues.

Les généralisations sur les résultats sont-elles adéquates?

Les conclusions sont-elles déduites des résultats?

La discussion est-elle limitée à l'interprétation et la signification de ces découvertes?

S'il y a des échappatoires dans l'interprétation, sont-elles reconnues? Devraient-elles être maîtrisées?

Devrait-on accepter d'autres interprétations?

Journal Fisheries Research Board Canada

# Guide for Typists Preparing Copy

Read the "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 8.5 × 11 inches (21.5 × 28 cm). If possible, use paper with the lines numbered in the margin. Leave all margins at least 1 inch (2.5 cm) wide. Use a nylon or carbon ribbon and keep the typewriter keys clean.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numbers. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send two copies (the ribbon copy and a carbon copy or a photocopy) to the Office of the Editor.

## Tables

Examine the current volume of the *Journal* to see how tables are set up (Appendix C).

Note especially the following concerning *numbered tables*:

Type each table on a separate page, using paper without the lines numbered.

Number the tables with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, they are typed with the text, they seldom need horizontal lines, and the data are arranged in as few rows as possible (Appendix C).

## Illustrations

Send the original and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

## Mathematical Expressions, Equations, and Formulas

Leave three spaces above and below lines that have much material in superscripts or subscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral one, and between the capital letter O and the numeral zero. Make other explanatory notes similarly.

## References

Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style that is used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list, but if an abbreviation is in doubt, type the name in full.

## Spellings

A list of about 100 words common in fisheries journals is given in Appendix F.

## Appendices

Appendices A to F are available on request from the Office of the Editor.

Journal Fisheries Research Board Canada

# Guide pour les secrétaires d'auteurs

Lisez le "Guide des auteurs" dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de taper ou d'arranger les détails, suivez la pratique du *Journal*.

Tapez le manuscrit sur un papier blanc et non un papier pelure, mesurant de préférence 8½ × 11 po. (21.5 × 28 cm) et laissant une marge d'au moins 1 po. (2.5 cm). Si possible, employez un papier avec des lignes numérotées dans la marge. Employez un ruban nylon ou carbone et gardez les clés du dactylo nettes.

Chaque partie du manuscrit doit être tapée à double-interligne au moins. La chose est particulièrement importante dans le cas des tableaux, légendes d'illustrations, renvois au bas de la page et listes de références bibliographiques. Evitez de souligner à moins que la matière ne soit en italique. Mettez en retrait la première ligne de chaque paragraphe du texte, ainsi que de chaque légende et renvoi.

Dans les titres et en-têtes, n'employez la majuscule que pour le premier mot, les noms propres et les adjectifs. Pour les petites majuscules, utilisez les petites lettres.

Indiquez tous les renvois au texte par des chiffres arabes en-tête. Numérotez-les consécutivement à travers le texte en commençant par ceux de la page-titre. Placez chaque renvoi immédiatement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit donner que le titre du sujet, le nom et l'affiliation de l'auteur ou des auteurs, et tout autre renvoi utile (Appendice A). Tapez le résumé sur une page séparée (Appendice B). Numérotez chaque page, débutant avec la page-titre.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et un double du travail au Directeur de la publication.

## Tableaux

Consultez une copie du *Journal* pour savoir composer les tableaux et placer les lignes qui y entrent (Appendice C).

Portez une attention particulière à ce qui suit concernant les *tableaux numérotés*.

Tapez les tableaux sur des pages séparées, en utilisant du papier sans lignes numérotées.

Numérotez les tableaux avec des chiffres arabes.

Employez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, et très rarement ailleurs. N'employez jamais de lignes verticales; de préférence, laissez de l'espace supplémentaire.

Indiquez les renvois dans les tableaux au moyen de petites lettres italiques en position supérieure et tapez-les au bas de la page.

Placez les tableaux après la liste de références.

A l'endroit où un tableau est mentionné pour la première fois, tapez dans la marge "Tableau (- - -) près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, ils sont tapés avec le texte. Ils ont rarement besoin de lignes horizontales, et l'information est préparée de façons à prendre le moins de rangées possibles.

## Illustrations

Joignez au manuscrit expédié le dessin original des illustrations et des photocopies en deux exemplaires.

Au bas et à gauche de chaque figure et photocopie, inscrivez le nom de l'auteur et le numéro de la figure. Aussi, tapez chaque légende avec sa figure pour fin d'identification. Pour les photographies, tapez l'information sur une lisière de papier et collez-la au dos de la photographie. N'écrivez pas et ne tapez pas sur la photographie même. Tapez ensemble sur une feuille séparée et par ordre consécutif les légendes des illustrations, et intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, tapez dans la marge "Fig. (- - -) près d'ici."

## Equations, formules et expressions mathematiques

Laissez trois espaces au-dessus et au-dessous des lignes qui contiennent du matériel en positions supérieures ou en positions inférieures, et au-dessus et au-dessous d'équations et de formules. Dans les différentes expressions, placez chaque composante dans sa position appropriée (Appendice D).

Indiquez les symboles clairement. Dans la marge, épelez les lettres grecques la première fois qu'elles apparaissent et faites une distinction entre la lettre "l" et le chiffre "un" et entre la majuscule "O" et le

chiffre "zéro." Procédez de la même façon pour d'autres notes explicatives.

### Références

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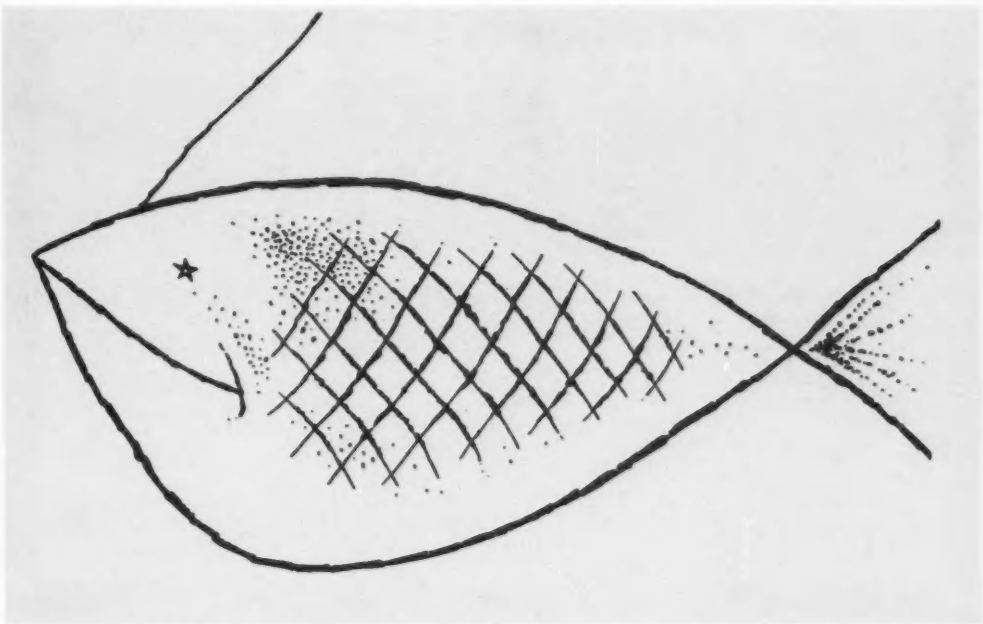

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